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GUSTAVO HENRIQUE TORRES DE ALMEIDA CAMILLO

FERMENTAÇÃO ÁCIDO LÁTICA DE SUCOS DE FRUTAS E VEGETAIS POR PROBIÓTICOS: ASPECTOS NUTRICIONAIS, SOCIAIS E ECONÔMICOS.

PROBIOTIC LACTIC ACID FERMENTATION OF FRUIT AND VEGETABLES JUICES: NUTRITIONAL, SOCIAL, AND ECONOMICAL ASPECTS.

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Dissertação apresentada a Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas para obtenção do título de Mestre em Alimentos e Nutrição.

Orientador: Prof. Dr. Mário Roberto Maróstica Junior

Coorientador: Prof. Dr. Juliano Lemos Bicas

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“O mais importante, é inventar o Brasil que nós queremos.” – Prof. Darcy Ribeiro

“Ninguém come PIB, come alimentos” – Profa. Maria Conceição Tavares

“A fome e a guerra não obedecem a qualquer lei natural, são criações humanas.” – Prof. Josué de Castro

RESUMO

Alimentos fermentados são produzidos há pelo menos 10 mil anos por diversas culturas espalhadas ao redor do globo. A técnica da fermentação de alimentos permite preservar o alimento, muitas vezes aumentando seu valor nutricional e funcional (ex: produção de vitaminas, aumento da atividade antioxidante pré-digestão de nutrientes, crescimento de microrganismos benéficos). Além disso, é facilmente implementada em pequena escala, pois não necessita de equipamentos de alta tecnologia nem grandes quantidades de energia. Nos últimos anos, um novo tipo de produto começou a ser desenvolvido, os sucos de frutas e vegetais produzidos por fermentação ácido lática controlada utilizando bactérias probióticas. Neste produto, uma bactéria probiótica utiliza o substrato do suco para se multiplicar, biotranformando a matriz alimentar, gerando um ambiente alimentar mais seguro em relação a microrganismos patógenos e gerando benefícios nutricionais. Dentre os principais benefícios nutricionais destes sucos destaca-se o aumento da atividade antioxidante, produção de diversos compostos bioativos e capacidade probiótica. Há uma infinidade de possibilidades de produção de sucos probióticos fermentados por bactérias ácido láticas variando o tipo de fruta ou vegetal, gênero, espécie e cepa da bactéria, tempo, temperatura, apenas para citar alguns parâmetros. Esta dissertação é composta por uma primeira parte que revisa os benefícios nutricionais dos sucos de frutas fermentados por bactérias ácido lácticas, e uma segunda parte que discorre sobre a fermentação de um suco de jabuticaba pela bactéria probiótica *Lactobacillus acidophilus*. A revisão explorou as diferentes possibilidades de produção de sucos e os possíveis compostos bioativos produzidos durante este processo, destacando os tipos de bactérias probióticas, tempo e temperatura da fermentação e a matriz alimentar. O resultado obtido na segunda parte foi um suco de jabuticaba probiótico (8 x 10^9 UFC na porção de 200 mL), com atividade antioxidante significativamente ($p<0.05$) maior comparada ao suco *in natura*. Ao fim, o trabalho concluiu pontos importantes do processo de fermentação ácido lática de sucos de frutas e vegetais dentro do contexto alimentar brasileiro. O processo de fermentação ácido lática pode ser aplicado a nível doméstico e em pequena escala, gerando benefícios econômicos a pequenos produtores, reduzindo perdas de produção, promovendo a biodiversidade brasileira e fornecendo alimentos de grande valor nutricional e funcional, tanto para os próprios produtores, quando para população em geral através da criação de novos mercados, incentivo ao comércio local e políticas públicas, em especial o Programa Nacional de Alimentação Escolar.

ABSTRACT

Fermented foods are being produced at least in the last ten thousand years by different cultures around the world. The food fermentation technique allows to preserve the food along with an increase of its nutritional and functional value (e.g., vitamin production, increased antioxidant activity, pre digestion of nutrients, beneficial microbes' growth.). Moreover, it is easily implemented on a small scale, as it does not require high-tech equipment or substantial amounts of energy. In the last years, a new type of product began to be developed, the fruit and vegetable juices produced by probiotic controlled lactic acid fermentation. In this product, a probiotic bacterium uses the juice substrate to multiply, bio transforming the food matrix, generating a safer food environment in relation to pathogenic microorganisms and also generating benefits. nutritional. Among the main health benefits of these juices, it can be highlighted the increase of antioxidant capacity, production of bioactive compounds and incorporation of probiotics. There is an infinity of possibilities to produce these juices varying the type of fruit and vegetable, genus, specie and strain of the microorganism, time, and temperature of fermentation, just to cite some parameters. This dissertation consists of a first part that reviews the nutritional benefits of fruit juices fermented by lactic acid bacteria, and a second part that discusses the fermentation of a jabuticaba juice by the probiotic bacterium *Lactobacillus acidophilus*. The review explored the different possibilities of production of juices and the possible bioactive compounds production during the process, featuring probiotic strain type, time and temperature of fermentation and food matrix. The result obtained in the second part was a probiotic (8×10^9 CFU in the portion of 200mL) jabuticaba juice, with significantly ($p<0.05$) higher antioxidant activity compared to fresh juice. In the end of the work, this dissertation concludes important points about the lactic acid fermentation of fruit and vegetables juices in the Brazilian food scenario. The lactic acid fermentation process can be applied at a domestic level and on a small scale, generating economic benefits for small producers, reducing production losses, promoting Brazilian biodiversity and providing food of great nutritional and functional value, both for the producers themselves, and for the general population through creation of new markets, incentive to local commerce, and public policies, especially the National School Food Program (PNAE).

ABSTRACTO

Los alimentos fermentados han sido producidos durante al menos 10 000 años por diferentes culturas alrededor del mundo. La técnica de fermentación de alimentos permite conservar los alimentos y aumentar su valor nutricional y funcional (p. ej., producción de vitaminas, aumento de la actividad antioxidante, predigestión de nutrientes, crecimiento de microorganismos beneficiosos). Además, es fácil de implementar a pequeña escala, ya que no requiere equipos de alta tecnología ni grandes cantidades de energía. En los últimos años se ha comenzado a desarrollar un nuevo tipo de producto, los jugos de frutas y hortalizas producidos por fermentación ácido-láctica controlada probiótica. En este producto, una bacteria probiótica (un microorganismo que administrado en cantidades adecuadas confiere efectos sobre la salud del huésped) utiliza el sustrato del jugo para multiplicarse, biotransformando la matriz alimentaria, generando un ambiente alimentario más seguro en relación con los microorganismos patógenos y generando también beneficios nutricionales. Entre los principales beneficios nutricionales de estos jugos se encuentra el aumento de la actividad antioxidante, la producción de varios compuestos bioactivos y la capacidad probiótica. Este producto puede ser una alternativa alimenticia rica em probióticos y vitamina B12 para poblaciones vegetarianas y vegetarianas estrictas. Hay una multitud de posibilidades para producir jugos probióticos fermentados por bacterias del ácido láctico, variando el tipo de fruta o verdura, género, especia y cepa de bacterias, tiempo y temperatura del proceso, solo por nombrar algunos parámetros. Esta disertación consta de una primera parte que revisa los beneficios nutricionales de los jugos de frutas fermentados por bacterias del ácido láctico, y una segunda parte que analiza la fermentación de un jugo de jabuticaba por la bacteria probiótica *Lactobacillus acidophilus*. La revisión exploró las diferentes posibilidades de producción de jugo y los posibles compuestos bioactivos producidos durante este proceso, destacando los tipos de bacterias probióticas, el tiempo y la temperatura de fermentación y la matriz alimentaria. El resultado obtenido en la segunda parte fue un jugo de jabuticaba probiótico (8 x 10^9 UFC en 200 mL del jugo), con una actividad antioxidante significativamente ($p<0.05$) mayor en comparación con el jugo in natura.. Al final, el trabajo concluyó puntos importantes del proceso de fermentación del ácido láctico de los jugos d frutas en el contexto alimentario brasileño. El proceso de fermentación del ácido láctico se puede aplicar a nivel doméstico y en pequeña escala, generando beneficios económicos para los pequeños productores, reduciendo pérdidas de producción, promoviendo la biodiversidad brasileña y proporcionando alimentos de gran valor nutricional y funcional, tanto para los propios productores como para la población en general a través de la creación de nuevos

mercados, incentivo al comercio local y de políticas públicas, en especial del Programa Nacional de Alimentación Escolar.

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1. INTRODUÇÃO GERAL

Os alimentos fermentados são definidos como alimentos ou bebidas produzidos através do desejável crescimento de microrganismos e conversão de componentes alimentares pela ação enzimática (Marco et al., 2021). A fermentação é uma técnica milenar de preservação de alimentos, encontrada em diferentes culturas ao redor do globo terrestre (Tamang et al., 2020), pois com o advento da agricultura há 10.000 anos atrás fez-se necessário as populações desenvolverem métodos de preservar os alimentos excedentes de suas produções para os períodos sem colheita. Após a secagem, a fermentação é o mais antigo método de preservação de alimentos conhecido, sendo sua descoberta possivelmente um acidente (Farnworth, 2003). Durante a fermentação, bactérias, leveduras e bolores produzem ácidos orgânicos, álcoois e metabólitos antimicrobianos que preservam este alimento do ataque de microrganismos patógenos e deteriorantes (Licandro et al., 2020; Marco et al., 2021). Esse crescimento seletivo de microrganismos benéficos fermentadores está associado a uma ampla gama de parâmetros físico-químicos a serem controlados durante o processo como temperatura, pH, atividade de água, presença ou não de oxigênio e potencial óxido-redutor do meio (Shiferaw Terefe and Augustin, 2020). Presentes em diversas culturas, estas técnicas de fermentação foram aperfeiçoadas com o tempo do ponto de vista nutricional, sensorial e no quesito da segurança dos alimentos, se tornando fontes estáveis de vitaminas, minerais, calorias e outros nutrientes, garantindo a segurança alimentar e nutricional destes povos (Tamang et al., 2020). Os alimentos fermentados abarcam diferentes dimensões científicas como a tecnológica, nutricional, econômica, antropológica e social, visto que na análise material histórica, essas dimensões não podem ser separadas para análise dos benefícios da fermentação para a vida do elemento humano.

Os alimentos fermentados contabilizam aproximadamente 1/3 do total de alimentos consumidos no mundo (Marco et al., 2021), chegando em algumas culturas africanas e asiáticas a 50% do valor calórico total dos indivíduos (Marshall and Mejia, 2011). Estas agriculturas milenares desenvolveram e aperfeiçoaram estes métodos de produção de alimentos fermentados, incorporando-os a sua cultura, transformando-os em alimentos básicos locais e criando um mercado local que gera renda e emprego aos trabalhadores da região (Ramos and Schwan, 2017). No caso brasileiro, é possível resgatar, principalmente de suas raízes africanas (Mokoena et al., 2016) e indígenas (Penna et al., 2017), ideias de produtos fermentados a serem produzidos, consumidos e comercializados por pequenos produtores rurais. Esta produção gera benefícios econômicos e nutricionais para a população rural, a partir da perspectiva da agroindústria familiar, modelo efetivo na promoção do desenvolvimento rural regional baseado na transformação de matéria-prima em produtos de maior valor agregado (Gazolla and Pelegrini, 2008). Não obstante, a fermentação é uma técnica barata (não necessita de equipamentos de alta tecnologia) e energeticamente eficiente de preservação de matérias-primas, sendo acessível às diversas regiões socioeconômicas do globo (Marshall and Mejia, 2011). Este processo é facilmente conduzido em pequena escala industrial ou até mesmo em uma escala doméstica de produção. Por exemplo, o Cauim, é uma bebida fermentada tradicional indígena brasileira feita a partir da mandioca, ocasionalmente adicionada de frutas (Noelli and Brochado, 1998). Nos últimos anos, essa bebida foi estudada pelo seu alto valor nutricional como grande atividade antioxidante, produção de vitaminas, microrganismos benéficos (Almeida et al., 2007; Ramos and Schwan, 2017). O aumento da produção destes tipos de bebidas fermentadas é de grande interesse, pois o incentivo ao consumo de alimentos fermentados está de acordo com as últimas evidências científicas devido ao seu grande valor nutricional (Marco et al., 2021), visto que há um movimento de cientistas a fim de incluir os alimentos fermentados nos guias alimentares ao redor do globo (Chilton et al., 2015).

A partir do marco microbiológico do isolamento de microrganismos no último século, abre-se um caminho aos alimentos fermentados, até então inexistente: a fermentação controlada de alimentos. Está técnica, que permitiu o aumento da produção de alimentos tradicionalmente fermentados como o pão, cerveja e vinho a partir do isolamento da levedura *Saccharomyces cerevisiae*, também abre portas para a criação de novos produtos até então nunca fermentados por nenhuma cultura ao redor do mundo. Neste quesito, nos últimos anos, houve um aumento expressivo nas pesquisas científicas sobre a fermentação controlada de sucos de frutas e vegetais por BAL probióticas (Garcia et al., 2020; Szutowska, 2020). Os probióticos são definidos como "microrganismos que quando consumidos em quantidades adequadas, conferem benefício à saúde do hospedeiro" (Hill et al., 2014) e alguns destes microrganismos são capazes de realizar a fermentação ácido-lática em sucos de frutas. Dentre estas bactérias capazes de realizar esse processo, destacam-se as BAL descritas dentre o antigo gênero *Lactobacillus*, hoje separado em 23 novos gêneros (Zheng et al., 2020), como também dos gêneros *Pediococcus, Streptococcus, Lactococcus, Weisella* e *Leuconostoc*. O resultado do produto da fermentação do suco depende, estritamente, não apenas do gênero e espécie da bactéria, como também da cepa desta (Garcia et al., 2020). Além do microrganismo, os parâmetros do processo fermentativo como tempo, temperatura, pH e substrato alimentar a ser utilizado influenciam diretamente no produto. Com isso, as possibilidades de produtos a serem desenvolvidos são vastas, principalmente no Brasil, país com a maior biodiversidade do mundo e rico em frutas nativas com grande potencial econômico e nutricional (Avila-Sosa et al., 2019; Clerici and Carvalho-Silva, 2011; Neri-Numa et al., 2018). Os benefícios nutricionais destes produtos, dependem de todos os parâmetros de processo acima, porém a incorporação de bactérias probióticas, produção de vitaminas e compostos bioativos e o aumento da atividade antioxidante são benefícios frequentemente relatados na literatura (Ayed et al., 2020; Szutowska, 2020). A **Figura 1**. resume o processo de fermentação ácido lática controlada por bactérias probióticas.

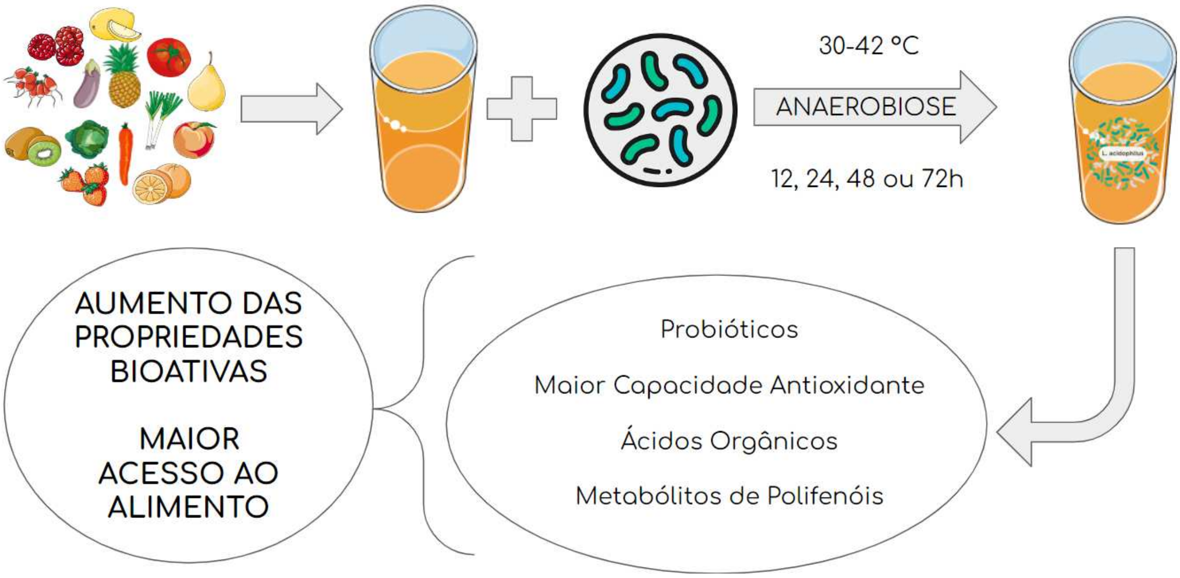

Figura 1. Resumo Gráfico da Fermentação Ácido Lática Controlada por Bactérias Probióticas.

Esta dissertação é composta por uma primeira parte que revisa os benefícios nutricionais dos sucos de frutas fermentados por bactérias ácido lácticas, e uma segunda parte que discorre sobre a fermentação de um suco de jabuticaba pela bactéria probiótica *Lactobacillus acidophilus*. Posteriormente, apresenta-se uma discussão geral sobre o papel da fermentação ácido lática controlada de sucos de frutas no quesito de sua aplicação prática. O capítulo de livro e o artigo original deste trabalho serão focados estritamente na questão nutricional e transformações bioquímicas da fermentação, porém a discussão geral trará justificativas da utilização deste processo a partir de outras áreas do conhecimento como a economia e a sociologia. Assim será justificado a partir de diferentes áreas do conhecimento científico que a produção de sucos probióticos ácido-lático fermentados utilizando frutas e vegetais brasileiros como matéria-prima é um processo que une a ciência e cultura e beneficia a população brasileira socialmente, nutricionalmente e economicamente.

2. REVISÃO DA LITERATURA

"Nutritional benefits of fruit and vegetable beverages obtained by lactic acid fermentation" será publicado em setembro no livro "*Value-Addition in Beverages though Enzyme Technology"* de Mohammad Hossain and Mohammed Kuddus, Elsevier (2022),"

Nutritional benefits of fruit and vegetable beverages obtained by lactic acid fermentation

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ABSTRACT: Fermentation is a traditional preservation technique that also improves the sensorial and health benefits of fruits and vegetables by the enzymatic machinery of the microorganisms, creating a high added-value product. Improved antioxidant capacity, production of health-related metabolites, incorporation of probiotic properties, and vitamin production are some of the benefits found in fermented fruits and vegetables beverages. In the last decade, the scientific and technological interest on fermented products raised due to their health and natural appeals to consumers. Moreover, the development of fermented fruits and vegetables beverages may be an alternative product to lactose–intolerant people and vegan consumers. Lactic acid fermentation of fruits and vegetables beverages by probiotic bacteria has increasingly been investigated in the past years due to the potential production of

"synbiotic" products, combining both polyphenols, non-digestible carbohydrates, and probiotics. Thus, this chapter focuses on the lactic acid fermentation of fruit and vegetables by probiotic bacteria highlighting their process conditions, health-related metabolites, and future perspectives.

KEYWORDS: Lactic Acid Bacteria, Fruits, Vegetables, Vitamins, SCFA, Probiotics, GABA, Antioxidant Capacity, Fermentation

Introduction

Since the advent of agriculture, human beings need to preserve the surplus of their production for times without harvest and one of the oldest techniques developed to preserve foods is through fermentation (Marshall e Mejia, 2011). Fermentation is an ancient preservation technique that improves shelf-life, taste, aroma, nutritional value, and palatability (Setta et al., 2020). This preservation technique was empirically sculpted during human evolution to maximize the technological, nutritional, and medical effects of fermented foods.

Fermented foods and beverages are defined as "foods made through desired microbial growth and enzymatic conversions of food components" (Marco et al., 2021). The consumption of these products accounts for approximately 1/3 of the total food consumed in the world, reaching up to 50% of the total caloric value of individuals in some African and Asian cultures (Marco et al., 2021; Marshall e Mejia, 2011). During fermentation, microorganisms produce alcohol, carbon dioxide, organic acids, antimicrobial peptides, and other metabolites that contribute to its microbial stability and microbial safety (Paul Ross et al., 2002). Moreover, fermentation, especially lactic acid fermentation, may enhance nutritional and technological aspects of a food product in a cost-effective and sustainable process, resulting in an added-value product (Marco et al., 2017). Furthermore, fermented foods are encouraged to be included in dietary guidelines around the world (Bell et al., 2017).

Fruits and vegetables are a great source of dietary fiber, phenolic compounds, carotenoids, vitamins, and minerals and preserve these nutrients and bioactive compounds using fermentation contributes to the food and nutrition security (Fusco et al., 2017). Thus, as the consumption of fruits and vegetables as part of a healthy diet is stimulated by the United Nations Food and Agriculture Organization (FAO) due to their prevent role on non-communicable chronic diseases (NCD's) (Food and Agriculture Organization of the United Nations (FAO), 2020), the development of techniques that prolong the shelf-life of these products and even improve their nutritional benefits are highly desirable.

Currently, the fermented beverages market is dominated by dairy products, such as yogurt and other fermented dairy beverages (Garcia et al., 2020). However, following the increasing demand for natural, healthier and more sustainable foods, the interest for lactic acid fermented beverages made from fruits and vegetables is likely to increase (Food and Agriculture Organization of the United Nations (FAO), 2018; Marshall e Mejia, 2011). In terms of health benefits, fermented fruits and vegetables beverages usually present, for instance, probiotics, antioxidants, short chain fatty acids, vitamins, and neuroactive compounds (Szutowska, 2020), as summarized in **Figure 1**.

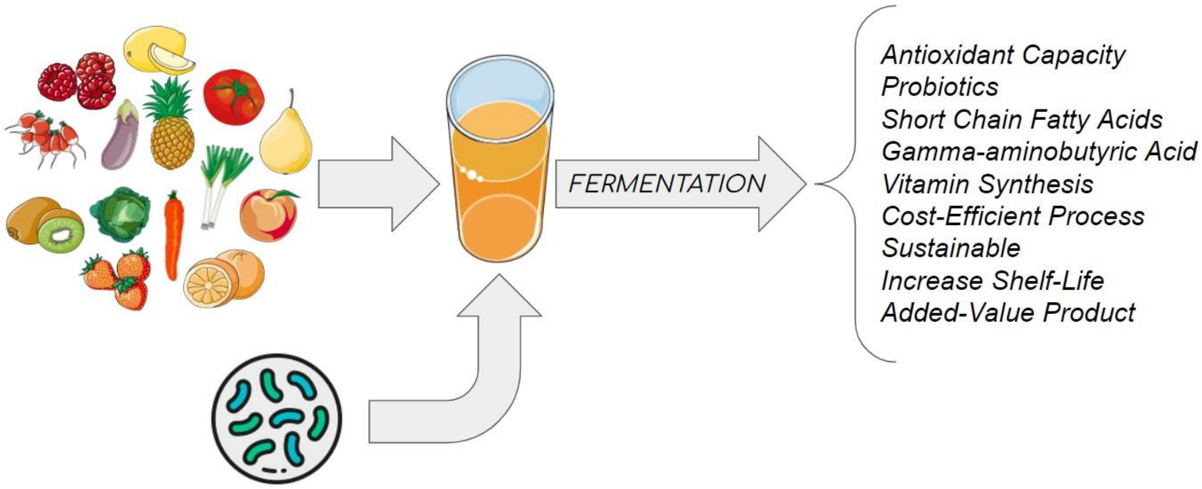

Figure 1. Benefits of lactic acid fermented fruits and vegetables beverages.

This chapter will cover the benefits and future perspectives in the manufacturing of new fermented fruit and vegetables beverages focusing on fermentations carried out by lactic acid bacteria. Thus, this chapter will present health benefits of lactic acid fermentation, and new lactic acid fermented fruits and vegetable beverages (NFB) developed in the last years with their nutrition improvements.

Lactic Acid Bacteria and Lactic Acid Fermentation

As the name says, lactic acid bacteria (LAB) is a group of gram-positive, non-spore-forming, catalase-negative, acid-tolerant bacteria producing lactic acid during the fermentation of carbohydrates, and include the genera *Aerococcus, Carnobacterium, Enterococcus, Fructobacillus, Lactobacillu*s (*L.*)*, Lactococcus, Leuconostoc, Oenococcus,* *Pediococcus, Streptococcus* (*S.*)*, Tetragenococcus, Vagococcus,* and *Weissella* (*W.*) (T, 2018). Most of the LAB strains produce a wide range of enzymes such as lipase, amylase, protease and glucoamylase that can break down food constituents such as proteins, carbohydrates and lipids and enhance absorption of these components compared to the original matrix (Mathur et al., 2020). The genus most related with the lactic acid fermentation of fruits and vegetables beverages is *Lactobacillus* that were reclassified in 2020 in 25 genera. The group of scientists suggest 23 new genera: *Acetilactobacillus, Agrilactobacillus, Amylolactobacillus, Apilactobacillus, Bombilactobacillus, Companilactobacillus, Dellaglioa, Fructilactobacillus, Furfurilactobacillus, Holzapfelia, Lacticaseibacillus, Lactiplantibacillus, Lapidilactobacillus, Latilactobacillus, Lentilactobacillus, Levilactobacillus, Ligilactobacillus, Limosilactobacillus, Liquorilactobacillus, Loigolactobacilus, Paucilactobacillus, Schleiferilactobacillus,* and *Secundilactobacillus* (Zheng et al., 2020). The new classification will be adopted throughout this chapter and the generic term "*Lactobacilli*" will be used to designate all organisms previous classified as *Lactobacillus*, as indicated previously (Zheng et al., 2020).

The LAB from *Lactobacilli* group and from the genera *Weisella, Leuconostoc, Enterococcus, Pediococcus* are widespread and identified in several traditional fermented plants, being *Lactiplantibacillus plantarum* the most common specie found in these products and used in the development of NFB (Penna et al., 2017; Szutowska, 2020). This specie is important in the technological development of fermented fruit and vegetable beverages because it produces pectinases, such as polygalactranose, pectinylase, and pectin esterase that promotes the sensorial aspects of the fermented juice (Devaki e Premavalli, 2019). Although not phylogenetically classified as LAB, *Bifidobacterium* genus is used for the production of the NFB because of their similar health metabolites production (Garcia et al., 2020).

Lactic acid fermentation could be done in three different methods. The first refers to the spontaneous fermentation, in which the endogenous microbiota of the fruits and vegetables grow in uncontrolled conditions. The endogenous microbiota of these foods are generally a consortium of microorganisms and each microorganism have their own time during the fermentation when their activity is high. This microbiota must have the capacity to grow fast and inhibit the grow of other pathogenic bacteria (Cuvas-Limon et al., 2020; Marshall e Mejia, 2011; Szutowska, 2020). The second method is the back-slopping. On this method, part of the previous batch is added to the new fermentation with the introduction of a food-adapted microorganism. This procedure accelerates the initial phase of fermentation and ensure the quality of the product as the core microbiota added tends to produce the same technological, nutritional, and sensorial effects on the product. Finally, the third type refers to the controlled fermentation using a well-known starter culture (Marshall e Mejia, 2011; Szutowska, 2020). This is the method used in the process of the NFB studied in the last years by the scientific community.

The spontaneous fermentation and the back-slopping method approaches are mainly applied at households and small farmer’s scale (Cuvas-Limon et al., 2020). The controlled fermentation is associated with the fermented products of the food industry in a high-scale production (Shiferaw Terefe e Augustin, 2020). Developed countries like Canada, USA, Australia, New Zealand and European countries usually utilize a well-defined starter culture on their fermented foods, while Asian and African countries usually consume their fermented foods made by spontaneous fermentation or back-slopping (Tamang et al., 2020).

The health benefits of the lactic fermentation of fruits and vegetables include the improvement of antioxidant capacity, the production of short-chain fatty acids, the incorporation of probiotic properties, and the increase in prebiotic and vitamin content, besides the production of neuroactive molecules for mental health (Devaki e Premavalli, 2019; Garcia et al., 2020; Szutowska, 2020). Therefore, the metabolism of each LAB in the biotransformation of food components of fruits and vegetables must be considered in the development of specific fermented products with health claims. The biotransformation of sugar, phenolic compounds, fibers, organic acids, amino acids into bioactive molecules depends not only on the specie of the microorganism, but also on the strain as well. Sivamaruthi et al. classified these bacteria strains that produce bioactive compounds as bioactive microorganisms (Sivamaruthi et al., 2018). The isolation of bioactive microorganisms from traditional fermented foods around the world (e.g., Cauim, Chicha, Kefir, Kombucha, and Togwa) is an effectively approach to obtain new strains with special metabolic characteristics and technological aspects for fermentation as utilization of carbohydrates sources, wide range of temperature growth, speed growth, and different metabolism pathways to the production of molecules of human nutrition interesting (Anukam e Reid, 2009; Pimentel et al., 2021; Satish Kumar et al., 2013).

Health Benefits of Plant-Based beverages produced by Lactic Acid Fermentation

This section is destinated to the health benefits and bioactive molecules produced during the lactic acid fermentation of fruits and vegetables. The **Table 1** shows some of the fruits and vegetable beverages produced by lactic acid fermentation in the past five years, highlighting the microorganisms, process parameters and nutritional benefits.

Table 1. New lactic acid fermented fruit and vegetable beverages, process parameters and main nutritional benefits (Note: ↑ and ↓ refers to "increase" or "decrease of", respectively). The name of the *Lactobacilli* is according to the new classification.

Fruit/Vegetables	Microorganism	Fermentation Conditions	Main Nutritional Benefits	References
Apple	*Lactiplantibacillus plantarum* ATCC14917	72h at 37°C Inoculum: 6.48 CFU/mL	↑ Total Phenolic and Flavonoid Content ↑ Antioxidant capacity (DPPH and ABTS from 0h to 24h and 48h to 72h of fermentation) ↓ Antioxidant capacity (DPPH and ABTS from 24h to 48h of	(Li et al., 2019)

			fermentation)	
Apricot	*B. lactis Bb-12* *B. longum Bb-46* *Lacticaseibacillus casei 01* *L. acidophilus LA5*	24h at 37°C Inoculum: 10^6 CFU/mL	Slightly ↑ Antioxidant capacity of the juice in all strains tested separately, except for *Lacticaseibacillus casei* 01 ↑ Acetate Production specially by LAB and in mixed fermentations	(Bujna et al., 2018)
Bergamot	*Lactiplantibacillus plantarum* AF1 *Lactiplantibacillus*	72h at 37°C Inoculum: 10^6 CFU/mL	All strains separately: ↑ Antioxidant capacity (DPPH) The combination of	(Hashemi e Jafarpour, 2020)

	plantarum LP3 *Lactiplantibacillus plantarum* subsp. *plantarum* PTCC 1896		the three strains produced the higher antioxidant capacity after 72h of fermentation.	
Blueberry	*Four strains of Lactiplantibacillus plantarum* *Five strains of Limosilactobacillus fermentum*	48h at 37°C Inoculum: 10^7 CFU/mL	All strains separately: ↑ Antioxidant capacity (ABTS, FRAP) ↑ Total Phenol Content specially by *Limosilactobacillus fermentum* strains	(S. Li et al., 2021)
Blueberry and	*Lactiplantibacillus plantarum* BNCC	48h at 37°C	↑ Antioxidant capacity (ABTS) in	(Wu et al.,

Blackberry	337796 *S. thermophilus* CGMCC 1.8748 *B. bifidum* CGMCC 1.5090	Inoculum: 5 × 10^8 CFU/mL	blueberry and blackberry juice by *Lactiplantibacillus plantarum* and *B. bifidum*	2021)
Cactus Pear	*Lactiplantibacillus plantarum* S-811	Fermentation at 37°C until the juice reaches a pH of 3.7 2% of inoculum 5.5 × 10^4 CFU/mL	↑ Acetate ↓ Body weight gain and fasting glucose in obese animal model	(Verón et al., 2019)
Cashew apple	*L. acidophilus* TISTR 1338	24h at 30°C Inoculum: 1.5 x	All strains separately: ↑ Prebiotics	(Kaprasob et al., 2018)

	Lacticaseibacillus casei TISTR 390 *Lactiplantibacillus plantarum TISTR 543* *Leuconostoc mesenteroides TTISTR 053* *B. longum TISTR 2195*	10^9 CFU/mL	↑ Vitamin B group ↑ Vitamin B12 specially with *L. plantarum*	
Cherimoya	*Levilactobacillus brevis* CRL 2050 *F. tropaeoli* CRL 2039 *Levilactobacillus brevis* CRL 2051 *Lactiplantibacillus*	48h at 30°C Inoculum: 10^7 CFU/mL	↑ Acetate by *Lb. brevis* CRL 2050 and *F. tropaeoli* separately ↑ Antioxidant capacity (DPPH) by *L. rhamnosus* and *F. tropaeoli* separately	(Isas et al., 2020)

	plantarum CRL 2030 *Lacticaseibacillus rhamnosus* CRL 2049		Maintenance of antioxidant capacity (ABTS) in fermented juice compared with non-fermented after 48h	
Coconut Water	*Lacticaseibacillus casei* L4	48h at 35°C Inoculum: 10^8 CFU/mL	↑ Antioxidant Capacity (DPPH, ABTS) ↑ Vitamin B12 ↑ Acetate	(Giri et al., 2018)
Coconut Water	*Lactiplantibacillus plantarum* DW12	72h at 37°C Inoculum: 10^7 CFU/mL	↑ Antioxidant Capacity (DPPH, ABTS) ↑ Vitamin B12 and	(Kantachote et al., 2017)

			B1 ↑ GABA	
Ginkgo Biloba Kernel	*Lactiplantibacillus plantarum BNCC 337796* *L. acidophilus BNCC 185342* *Lacticaseibacillus casei ATCC 393*	48h at 37°C Inoculum: 10^7 CFU/mL	All strains separately: ↑ Antioxidant capacity (ABTS, FRAP)	(Wang et al., 2019)
Jujube	*L. acidophilus 85* *Lactiplantibacillus plantarum 90*	48h at 37°C Inoculum: 10^7 CFU/mL	All strains separately: ↑ Antioxidant capacity (DPPH	(T. Li et al., 2021)

	L. helveticus 76 *Lacticaseibacillus casei 37*		and FRAP)	
Kanji (Black Carrot)	*Pediococcus acidilactici* BC1	10d at 37°C Inoculum: 8 × 10^7 CFU/mL	↑ Antioxidant capacity (ABTS) ↑ Phenolic Content ↑ Antibacterial activity against *S. boydii* and *S. enterica*	(Manzoor et al., 2021)
Litchi	*Lacticaseibacillus casei* CICC 6117	24h at 30°C Inoculum: 10^6 CFU/mL	↑ Antioxidant capacity (ORAC)	(Wen et al., 2020)

Mulberry	*Levilactobacillus brevis* F064A	48h at 37°C Inoculum: 10^8 CFU/mL	↑ GABA ↑ Antioxidant Capacity (DPPH) ↑ Antibacterial activity	(Kanklai et al., 2021)
Mulberry	*Lactiplantibacillus plantarum ATCC SD5209* *L. acidophilus ATCC SD5212* *Lacticaseibacillus paracasei* *ATCC SD5275*	36h at 37°C Inoculum: 10^5 CFU/mL	All strains separately: ↑ Antioxidant capacity (DPPH, ABTS, RP-CA) *Lactiplantibacillus plantarum* exhibited higher enhance on antioxidant	(Kwaw et al., 2018)

			capacity	
Papaya	*Lactiplantibacillus plantarum GIM1.140* *L. acidophilus GIM1.731*	48h at 37°C Inoculum: 5% of the mass ratio of the fermentation broth	All strains separately: ↑ Flavonoids content ↑ Acetate specially with *Lactiplantibacillus plantarum* *L. acidophilus*: ↓ Antioxidant capacity (DPPH, ABTS, FRAP, CUPRAC) *Lactiplantibacillus plantarum*: ↑ Antioxidant capacity (CUPRAC, FRAP,	(Chen et al., 2018)

			DPPH)	
Pineapple	*L. acidophilus LA5* *B. lactis Bb12* *Lactiplantibacillus plantarum 299V*	24h at 37°C Inoculum: 10^6-10^7 CFU/mL	All strains separately: Slight ↑ Antioxidant capacity (FRAP) Slight ↑ Phenolic Content ↑ Acetate	(Nguyen et al., 2019)
Pomegranate	*Lactiplantibacillus plantarum* ATCC14917	24h at 30°C Inoculum: $10^{11.42}$ CFU/mL	↑ Antioxidant capacity (ABTS) ↑ Acetate production during storage at 4°C for 4 weeks	(Mantzourani et al., 2019)

Pomegranate	*Lactiplantibacillus plantarum* PU1	24h at 30°C 2×10^7 CFU/mL	↑ Antioxidant capacity (DPPH, ABTS) ↓ Linoleic Acid Peroxidation	(Pontonio et al., 2019)
Pomegranate	*L. acidophilus CECT 903* *Lactiplantibacillus plantarum CECT 220* *B. longum subsp. infantis CECT 4551* *B. bifidum CECT 870*	24h at 37°C Inoculum: 10^6 CFU/mL	All strains separately: ↑ Antioxidant capacity (DPPH, ABTS)	(Valero-Cases et al., 2017)

Strawberry and Blueberry	*Levilactobacillus brevis* CRL 2013	7d at 30°C Inoculum: 5 × 10^7 CFU/mL	Anti-inflammatory effect in mice model ↑ GABA, especially after 24h of fermentation and when the juice was supplemented with yeast extract.	(Cataldo et al., 2020)
Sweet Lemon	*Lactiplantibacillus plantarum* LS5	48h at 37°C Inoculum: 10^9 CFU/mL	↑ Antioxidant capacity (DPPH and FRAP) ↑ Antibacterial activity	(Hashemi et al., 2017)

Tomato	*Lactiplantibacillus plantarum* *Lacticaseibacillus casei* (strains not informed)	24h at 37°C (Initial concentration not informed)	All strains: ↑ Antioxidant capacity (ABTS, FRAP, DPPH) *Lactiplantibacillus plantarum*: ↑ Acetate	(Y. Liu et al., 2018)

Antioxidant Capacity

Interest in vegetable antioxidant compounds increases due to their protective effect on NCDs [2]. The phenolic compounds content in fruits and vegetables are the major contributor to their antioxidant activity. The basic antioxidant action of phenolic compounds in combating NCDs, for instance, is the scavenging of reactive oxygen species (ROS) present in the human body (Gulcin, 2020; Shahidi e Ambigaipalan, 2015). This sequestration inhibits the action of these ROS in cascades of cell signaling, thus inhibiting the production of inflammatory cytokines, and disfavoring the environment for the development of NCDs (Welty et al., 2016). Also, phenolic compounds can chelate transitional metal inactivating its catalysts and scavenge singlet oxygen, that contributes to the formation of ROS and consequently inflammation in human body and development of NCDs (Shahidi e Ambigaipalan, 2015).

Phenolic compounds in plants can be found in free form in the vacuoles of plant cells or bound to plant cell wall structure components (e.g., cellulose, hemicellulose, pectin, and lignin) (Huynh et al., 2014). The action of cellulolytic, ligninolytic and pectinolytic enzymes produced by the microorganisms during fermentation can release bound phenolics previous linked to the plant cell wall structure, increasing their free form, absorption, as well the antioxidant activity (Huynh et al., 2014). Also, lactic acid fermentation of plants are related to the biotransformation of phenolic compounds by enzymes such as phenolic acid reductase, β-glucosidase, tannase, esterases, benzyl alcohol dehydrogenase and phenolic acid decarboxylases (Rodríguez et al., 2009; Shiferaw Terefe e Augustin, 2020) . As one of the most common specie in the microbiota of plants, *Lactiplantibacillus plantarum* evolved over time to be able to degrade phenolic compounds found in high amount in these substrates (Rodríguez et al., 2009). There are two major mechanisms by which this specie and the majority of LAB could enhance the antioxidant activity of plant-based fermented beverages. The first one is the degradation of tannins by tannases and decarboxylases, forming gallic acid and pyrogallol that are powerful antioxidants compounds (Rodríguez et al., 2009). The second one is the biotransformation of glycosylated phenolics by β-glucosidase, resulting in an increase of their aglycone counterparts, with higher radical scavenging effect (Mousavi et al., 2013; Rodríguez et al., 2009). The glycosylation interferes with the phenolic ability to delocalize electrons, affecting its antioxidant activity (Heim et al., 2002). The **Figure. 2** shows the main enzymes, substrates and products related to the enhancing of antioxidant capacity by lactic acid fermentation.

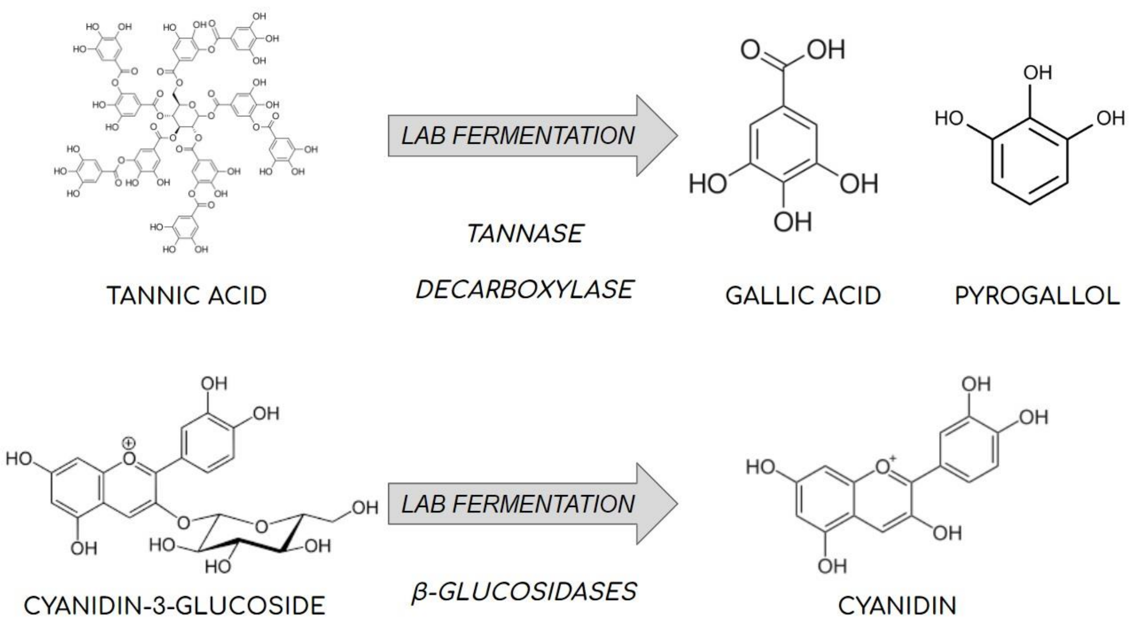

Figure 2. Biotransformation of phenolic compounds by lactic acid fermentation enhancing antioxidant activity.

The increase in antioxidant capacity by fermentation is observed in different studies using plant foods as substrate, in particular fermented beverages, or fruit juices (Szutowska, 2020). Thus, many articles show that LAB strains and *Bifidobacterium* can enhance antioxidant capacity of plant-based fermented beverages, such as the examples presented next.

The effect of fermentation on the antioxidant capacity of a cranberry juice using BAL was investigated (Kwaw et al., 2018). The juice was fermented for 36h at 37°C using

Lactiplantibacillus plantarum, *L. acidophilus* and *Lacticaseibacillus paracasei species*. The fermented juices showed higher antioxidant capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and reducing power capacity (RP-CA) methods compared to the non-fermented (control) juice. Along with the increase in antioxidant capacity, an increase in total phenolic compounds was observed ranging from 1035.81 ± 4.07 μg/mL in the control juice to 1516.08 ± 5.53, 1661.36 ± 6.27 and 1644.28 ± 6.07 μg/mL after fermentation with *Lacticaseibacillus paracasei, Lactiplantibacillus plantarum* and *L. acidophilus,* respectively (Kwaw et al., 2018). Similarly, Li et al. also reported the increase in the antioxidant capacity (DPPH method) of an apple juice fermented by another strain of *Lactiplantibacillus plantarum* (Li et al., 2019).

In another study involving the effects of LABs on the antioxidant capacity (DPPH and the FRAP methods) of juices, the effect of fermentation of a sweet lemon juice using the probiotic bacterium *Lactiplantibacillus plantarum* LS5 was analyzed (Hashemi et al., 2017). The antioxidant capacity of the fermented juice increased significantly compared to the unfermented juice used as the control in the study. Differently to study done by Kwaw et al. (2018), a decrease in total phenolic compounds was observed, although the level of ascorbic acid was maintained in the fermented juice (Hashemi et al., 2017).

The effect on antioxidant capacity after the fermentation of a pomegranate juice (rich in phenolic compounds, particularly anthocyanins) using *L. acidophilus* and *Lactiplantibacillus plantarum* separately was also evaluated. The fermentation occurred in anaerobiosis at 30°C for 72h. An increase in DPPH-scavenging effect was observed the juices fermented by either *L. acidophilus* (90%) or *L. plantarum* (75%) compared to the non-fermented juice (60%). The microorganisms managed to increase the antioxidant capacity mainly by hydrolyzing the polyphenols, producing their aglycone forms, which present higher values in the DPPH test (Mousavi et al., 2013). In another study, scientists reported that a litchi juice fermented by *Lacticaseibacillus casei* at a temperature of 30°C for 24h presented a significant increase ($p<0.001$) in antioxidant capacity (ORAC method) (Wen et al., 2020).

The fermentation of cherimoya juice using different strains of LAB was also studied in terms of increase in the antioxidant capacity (DPPH, ORAC, Trolox Equivalent Antioxidant Capacity (TEAC) methods). The samples were fermented 48h at 37°C. Although, an increase in antioxidant capacity was not evidenced in some of the fermented juices, the antioxidant capacity of the non-inoculated juice decreased after the same period, indicating that fermentation was important to at least to preserve the antioxidant capacity originally present in the unfermented juice (Isas et al., 2020).

Thus, the studies mentioned in this section indicate that the lactic acid fermentation of a juice rich in phenolic compounds by LABs generally increases their antioxidant capacity and this effect should improve the action of such fermented juices in the prevention of NCDs. The next sections will also present the increase in other bioactive molecules which could also contribute to health parameters.

Increase in Short Chain Fatty Acids

Acetate, propionate, and butyrate are three short chain fatty acids (SCFA) produced by the gut microbiota during the fermentation of non-digestible carbohydrates and have a local and systemic positive effects in the human body (Den Besten et al., 2015). Locally, the production of these SCFA lower the pH of the colon creating a better environment to the growth of beneficial bacteria at the expense of pathogenic bacteria. SCFA production in the gut is a biomarker of gut health and has been related with an increase of the immunity of the host, possibly preventing from infectious diseases, such as COVID-19 (Dhar e Mohanty, 2020). Other systemic effects of SCFA include, for example, the improvement of the intestinal barrier function, the regulation of lipid and glucose metabolism, protecting against weight gain, the stimulation of the production of satiety peptides, such as GLP-1 and PYY, besides the promotion of insulin secretion and adipose tissue browning, improving energy expenditure (Blaak et al., 2020).

These SCFA are formed by fermentation of gut microbiota and present in food products because of LAB fermentation. These SCFA are final catabolites of the LAB's energy metabolism and are produced by the fermentation of pyruvate and in heterofermenting conditions by the phosphoketolase route (Pessione, 2012). Although food fermentation can increase the amount of SCFA compared to unfermented products, there is a limited number of studies focusing on this SCFA production in fermented foods as well the biological effects of exogenous SCFA (Annunziata et al., 2020).

Acetate is a two-carbon SCFA produced by the gut microbiota and in food fermentation by acetic bacteria and some heterofermentative LAB. This acid is a primary product of the acetic fermentation and the major component of vinegar (Xia et al., 2020). Propionate is a three-carbon SCFA produced by some bacteria in the gut, but also by some LAB in food fermentation. Butyrate is a four-carbon SCFA produce specially in the gut by the species *Faecalibacterium prausnitzii, Eubacterium rectale*, *Eubacterium halii* and *Roseburia* *spp*. that ferment dietary fibers to yield this acid (H. Liu et al., 2018). Some LAB can produce butyrate during the fermentation of fruits and vegetables. *L. acidophilus, L. rhamnosus, L. bulgaricus and Lactiplantibacillus plantarum* are reported in the literature to increase the butyrate content during the fermentation of fruits and vegetables, due to the content of sugar and fibers in the food matrix (Annunziata et al., 2020).

Although LAB are not major producers of butyrate, the lactic acid and acetate produced by these bacteria can be used as substrate in the gut microbiota by another butyrate-producing bacteria in a crosstalk effect. The production of lactic acid and acetate stimulates the production of butyrate by these bacteria in the gut. So choosing the right specie and strain of LAB, the fermentation of fruits and vegetables could produce acetate, propionate, butyrate and lactic acid, which would be further mainly converted to butyrate in the gut (Bourriaud et al., 2005).

Most of the studies dealing with the fermentation of juice do not focus on the production of these SCFA, although some describe the production of mainly acetate, only in terms of simple chemical analyses, not evaluating its bioactive properties (Filannino et al., 2013; Mousavi et al., 2013). In the next paragraph, examples of reports dealing with SCFA during fermentation of plant-based beverages will be presented.

The production of SCFA in fermented foods and beverages was reviewed in a recent publication (Annunziata et al., 2020). In this study, the authors mention that a carrot juice fermented by *Lacticaseibacillus rhamnosus* presented an increased amount of SCFA compared to the non-fermented juice. Moreover, the fermentation of guava fruit by *Lactiplantibacillus plantarum* also improved the content of butyrate by more than 10 times.

This study also cited that even a short fermentation period (with four different LAB separately: *Lactiplantibacillus plantarum, L. acidophilus, Lacticaseibacillus rhamnosus* and *Lactobacillus delbrueckii subps. bulgaricus*) of black tea ranging from 7 minutes to 180 minutes could significantly enhance butyrate content in all lactic acid strains tested compared to the non-fermented tea that presented butyrate in non-detectable content. Finally, this review also mentioned that kombucha (with or without pollen addition) fermentation by the Symbiotic Culture of Bacteria and Yeasts (SCOBY) could also improve the content of acetate, propionate and butyrate compared to the non-fermented kombucha. The last example is important to the context of this chapter since SCOBY has a considerable amount of LAB in its composition (Sinir et al., 2019). Similarly, Isas et. al reported the increasing of acetate levels during the fermentation of a cherimoya juice utilizing a wide variety of *lactobacilli* strains , while the fermentation of a carrot juice by the probiotic bacteria *Lacticaseibacillus rhamnosus* GG significantly increased the content of acetate, propionate and butyrate compared to the unfermented juice (Hu et al., 2019).

Incorporation or increase in Probiotic, Prebiotic and Synbiotic properties

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Hill et al., 2014) Some beneficial health effects inherent to probiotics are shared among all microorganisms considered to be probiotics, some effects only between the same species and other more specific effects of some strains. *Lactobacilli* and *Bifidobacterium* are the two genera with most strains considered as probiotic. The positive effects attributed to probiotics include the production of SCFA and other acids, protection against colon colonization by pathogenic microorganism, restoration of a healthy intestinal microbiota, exclusive competition with pathogens, and the promotion of turnover of enterocytes (Hill et al., 2014; Parvez et al., 2006). Currently, most starter cultures used to produce lactic acid fermented fruits and vegetables beverages under controlled conditions are probiotic bacteria (Szutowska, 2020). In this case, if the final microbial count reaches 10^9 CFU/ml, the resulting beverage could be considered probiotic (Hill et al., 2014).

As claimed by other authors (Marco et al., 2020), the dietary consumption of nonharmful or commensal live microorganisms could be beneficial to the human health even without the specific characterization of the strain level. Fermented foods that are not further treated to reduce microbial load (e.g. pasteurization) could provide this daily intake of live microorganisms, such as observed for ancient diets compared to the current western industrialized diet (Rezac et al., 2018). Therefore, dietary intake of these microbes is one of the benefits of the lactic acid fermented fruits and vegetables beverages.

The definition of prebiotics was first conceived in 1995 by Gibson and Roberfroid when they analyzed some carbohydrates capable of promoting the growth of intestinal bacteria of the *Lactobacilli* and *Bifidobacterium*, thus promoting beneficial health effects (Gibson e Roberfroid, 1995). This definition was updated periodically and, in 2017, the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined prebiotic as "a substrate that is selectively utilized by the gut microbiota thus conferring health benefits" (Gibson et al., 2017). With this new definition, not only non-digestible carbohydrates can be considered prebiotics but also any other substance that when metabolized by the gut microbiota confers a beneficial health effect. Food substances that have been extensively studied as prebiotics since the ISAPP definition are the phenolic compounds. Most phenolic compounds present in plants are not absorbed by the human gastrointestinal tract, being metabolized by the intestinal microbiota when they reach the colon. This biotransformation generates health benefits in two distinct ways: generation of phenolic compounds with higher antioxidant capacity and bioavailability (Corrêa et al., 2019) and growth of specific bacteria of the gut microbiota related to good health markers, such as *Akkermansia muciniphila* in the case of flavonoids (Anhê et al., 2016).

Most plant-based foods, such as fruits, vegetables, spices, legumes, teas, and whole grains, contains a combination of prebiotic carbohydrates (oligosaccharides) and a range of polyphenols and other phytochemicals that can modulate the gut microbiota (Cao et al., 2019). Some people do not have an adequate gut microbiota to metabolize the dietary fiber (Gill et al., 2021) and the phenolic compounds (Bento-Silva et al., 2020) present in these plant-based foods, so lactic acid fermentation of these foods and beverages could be a tool to enhance the beneficial health effects in this population, improving the absorption of metabolized phenolic compounds (Bento-Silva et al., 2020) and SCFA, but more studies are needed.

When a product combines both probiotic and prebiotic claims, it may be considered "synbiotic". Synbiotics are defined as a mixture of live microorganisms and substrates selectively used by host microorganisms that confer health benefits (Swanson et al., 2020). In 2020, two terms related to the "synbiotic" classification have been developed. The so-called synergistic synbiotics were defined as a mixture of a microorganism and a substrate where this substrate is selectively used by the microorganism administered together. As for complementary symbiotics, the selected prebiotic is not used by the microorganism administered together, but by the host's intestinal microbiota (Swanson et al., 2020). Recently, Sharma and Padwad (2020) also classified the combination of polyphenols and probiotic bacteria as "second generation symbiotics", considering that the biotransformation of these metabolites generates new compounds with synergistic effects between health benefits and the probiotic bacteria itself (Sharma e Padwad, 2020). A plant-based beverage rich in phenolics fermented with a probiotic bacteria is a good candidate to be a second generation symbiotic.

One of the benefits in the production of fermented foods with *Lactobacilli* probiotic bacteria is their well-known capacity of enhance the vitamin content of the product (Leblanc et al., 2011), that will be described in next section.

Vitamin Synthesis

Most of the microorganisms cannot produce several vitamins, however some LAB and *Bifidobacterium* spp. can convert some dietary compounds into vitamin K and vitamins from the B complex, such as thiamine, riboflavin, niacin, pyridoxine, folate and cobalamin (Leblanc et al., 2011). Fermentation was one of the primary ways to ensure their vitamin daily intake in ancient cultures, since fermented foods have a vitamin content several times higher than the unfermented matrix (Marshall e Mejia, 2011).. This may be particularly important for vegans and pregnant women, for which cobalamin (vitamin B12) and folate, respectively, are especially important. The production of these two vitamins in fermentations by lactic acid bacteria will be presented in the following paragraphs.

Vitamin B12 cannot be produced by animals, plants or fungi, but it can be synthesized by the rumen microbiota of some animals, being primary produced by a few of anaerobic bacteria and archaea (Leblanc et al., 2011). The few anaerobic bacteria able to produces B12 include some LAB, such as *Limosilactobacillus reuteri* and *Lactiplantibacillus plantarum* (Leblanc et al., 2011). Therefore, the production of plant-based fermented beverages using lactic acid bacteria may be useful not only for its suitability to vegan people (a rising trend), but also to contribute to avoid cobalamin (vitamin B12) deficiency in this population. Kwak et al. evaluated the vitamin B12 intake of centenarians in South Korea and concluded that kimchi and other soybean-based traditional fermented foods contributes to the low incidence of vitamin B12 deficiency in these people compared to centenarians in western countries (Kwak et al., 2010).

The fermentation of cauliflower and white beans with the different strains of *Lactiplantibacillus plantarum* was analyzed. All strains analyzed significantly increased the content of vitamin B12 during 44h of fermentation at 30°C (Thompson et al., 2020). Also, another group of researchers described the production of a novel product, a guava gummy jelly, that uses as ingredient a fermented guava pulp by *Lactiplantibacillus plantarum* WU-P19. The fermentation of the pulp during 36h at 37°C increased the concentration of vitamin B12 in the final product and the guava gummy jelly product presented the total daily requirement of vitamin B12 in only 1g of the product (Palachum et al., 2020). Giri et al. analyzed the fermentation of coconut water beverage by *Lacticaseibacillus casei* L4 for 48h at 35°C. They find a significantly increase in extra-cellular B12 levels among others health improving benefits as antioxidant capacity, antimicrobial activity, and acetate production (Giri et al., 2018). In another study focusing on the fermentation of coconut water, researchers utilized *Lactiplantibacillus plantarum* DW12 for the fermentation process and related a 44-fold increase in vitamin B12 content after 48h of fermentation. As well, the researchers described maximal content of probiotic bacteria, antioxidant capacity, antibacterial activities, and GABA production after 48h of fermentation (Kantachote et al., 2017). The results showed are promising in the area, fermented fruits and vegetables beverages could ensure the vitamin B12 daily intake in restrict vegetarian diets with low amounts of the products. More studies should be done to analyses the vitamin B12 content of the lactic acid fermented fruits and vegetables.

Folate is an essential vitamin for one-carbon reactions, such as in the metabolism of amino acids and nucleotides. This is a critical vitamin in women pregnancy and breastfeeding, that encouraged de implementation of some public health practices, like enrichment of wheat flour in different countries (Viscardi et al., 2020). Moreover, thermal treatments may reduce the folate contend in foods, while fermentation may enhance folate concentrations (Jägerstad et al., 2004). Some strains of the species *L. delbrueckii subsp. lactis, Streptococcus thermophilus, L. acidophilus, Lactiplantibacillus plantarum, Lactococcus lactis* and others, for example, can produce folate during fermentation (Saubade et al., 2017), while some *Lactobacilli* do not have the genes involved in folate biosynthesis, and they can actually decrease the initial folate content (Leblanc et al., 2011). This ability to produce folate was also evaluated in a study with 55 strains of the *Lactobacilli* group. The results revealed that from the eight strains of *Lactobacillus acidophilus* analyzed, only two were capable to produce this vitamin. On the other hand, from the 18 strains of *Lactiplantibacillus plantarum* tested, 15 produced folate. Among all microorganisms investigated, the major folate producer was one strain of *Lactobacillus amylovorus* (Laiño et al., 2014).

A limited number of articles analyzing the production of folate during the lactic acid fermentation of fruits or vegetables juices is available in literature. As summarizes Saubbade et al., the folate enrichment by lactic acid fermentation in milk products is well known, but scarce in fruits and vegetables matrices (Saubade et al., 2017). Some of the examples are shown next. The folate level before and after fermentation of cucumber and watermelon juice by *Lactococcus lactis cremoris* were analyzed. The initial folate content was 10 ± 0.2 and 18

± 0.9 ng/ml in cucumber and watermelon extracts, respectively. Fermentation improved the final folate content of cucumber and watermelon extract to 60 ± 1.9 and 26 ± 1.6 ng/ml respectively (Gangadharan e Nampoothiri, 2011). Four strains of *Lactiplantibacillus plantarum* in the fermentation of a mixture of white beans and cauliflower at 30°C for 44h were tested and was observed an enhanced folate levels in all samples, reaching 58.8 ± 2.0µg/100 g fresh weight with the strain *L. plantarum* 299v, a 60% increase comparing with the initial value (Thompson et al., 2020). The fermentation of orange, apple and grape juices by *Lactobacilli* strains was also evaluated for folate production. Folate production was only observed in the apple juice fermented by strains of *L. plantarum and L. rhamnosus*. However, the amount of folate produced (maximal of 1.75µg/100 ml) was not considered high enough to affirm that this product could be a source of this vitamin, reaching in the two juices fermented (Espirito-Santo et al., 2015). In combination, these two studies may suggest that vegetables are a better matrices than fruits when the objective is to developed folate-enriched products by fermentation with *Lactiplantibacillus plantarum* 299v.

This section discussed that vitamin-enriched juices fermented by lactic acid bacteria could be achieved and this strategy may help to ensure the daily intake of these nutrients. However, it was evident that an appropriate strain selection is important to achieve and adequate production of these vitamins. In this sense, *Lactiplantibacillus plantarum* is of particular interest, because it can produce both folate and cobalamin in significant amounts.

Improving Mental Health

Anxiety and depression rates are increasing in the XXI century due to a complex of biological and sociological reasons (World Health Organization (WHO), 2017). As for the former food intake may contribute to a desired balance , such as γ-aminobutyric Acid (GABA), serotonin, dopamine and melatonin, which are synthesized by some plants and microorganisms and are found in a great number of fermented foods (Yılmaz e Gökmen, 2020). In fermented fruits and vegetables, the production of GABA is the most notorious (Yılmaz e Gökmen, 2020).

GABA is the main inhibitory neurotransmitter in the brain and its dysfunction in the body is related to the depression and anxiety (Dinan et al., 2013). GABA also have other health benefits as lowering cholesterol, hypotensive effect and promoting insulin secretion (Li e Cao, 2010). The human body can obtain GABA through the lactic acid fermentation of glutamate by the gut microbiota (**Figure 2**). Using the gut-brain axis, the GABA produced by the lactic acid bacteria in the gut reach the brain and alleviate the effects of depression and anxiety. Studies have shown that the production of GABA can be done under the lactic acid fermentation process of the fermented foods and beverages (Li e Cao, 2010). GABA is a molecule produced by the LAB utilizing the enzyme GAD, glutamate decarboxylase, and its coenzyme pyridoxal-5'-phosphate (P5P) (Figure xxx). A great variety of LAB species can produce glutamate decarboxylase, such as *Lactobacilli*, *Streptococcus* spp., and *Lactococcus* spp. Several studies indicate the production of GABA in some traditional fermented vegetables and cereals (e.g., *kimchi*, *paocai*, *marcha sikkim*) made with a wide range of LAB species such as *Levilactobacillus brevis*, *Lactobacillus delbrueckii* subps. *lactis, Lentilactobacillus buchneri,* and *Enterococcus faecium* (Yogeswara et al., 2020). Similarly, a mulberry juice fermented by *Levilactobacillus brevis* F064A had a GABA concentration increased from 0.1mg/mL (non-fermented juice) to 3.31mg/mL (fermented juice). The strain used in this study was isolated from a traditional fermented food from Thailand, Sai Krok Isan, a fermented sausage (Kanklai et al., 2021).

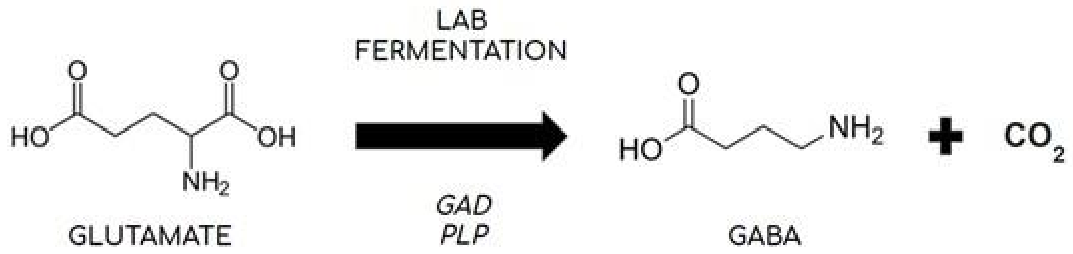

Figure 2. Biotransformation of glutamate into GABA by LAB.

In recent years, it became evident that gut microbiota impacts mental health by the so-called gut-brain axis (Cryan e Dinan, 2012). The gut-brain axis is a communication between the central and enteric nervous system and the peripheral intestine. This axis links emotional and cognitive patterns of the brain and intestinal functions, in which gut microbiota plays a crucial role (Carabotti et al., 2015). Therefore, the administration of the probiotic itself might be beneficial to the health mental status. In this case, such probiotics with a positive impact to mental health are called psychobiotics, which are defined as a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness (Dinan et al., 2013). A wide range of LAB are considered psychobiotic not only due to the production of GABA, but other neurotransmitters as well. For example, the genera *Streptococcus* and *Enterococcus* can produce serotonin in the gut (Dinan et al., 2013). Therefore, a proper selection of a LAB strain and the establishment of the right fermentation conditions may assure the endogenous production of GABA, dopamine, serotonin, and other neuroactive peptides that can regulate functions and behaviors of the central nervous system, benefiting clinical conditions such anxiety, daily stress, fatigue, depression, and sleep quality (de Araújo e Farias, 2020). Moreover, as previously mentioned, the isolation and identification of LAB strains present on traditional fruits and vegetables foods and beverages could be a strategy to discover to new candidates of psychobiotic strains.

Conclusion and Future Perspectives

Fermented plant-based beverages made using LAB are added-value products with great commercial potential and marketing appeals due to their health, technological and sensorial benefits improved during fermentation. The use of the enzymatic machinery of the microorganisms is a sustainable and cost-efficient process that can be applied both in industrial and home levels. Thus, the biodiversity of native fruits and vegetables in different countries, as well as the variety of lactic acid bacteria and their different metabolisms create and infinity of possibilities to scientists and industries to explore. As addressed in this chapter, *Lactiplantibacillus plantarum* is the most common specie related with traditional fermented beverages and with the health benefits of lactic acid fermented juices. More research is needed in the isolation of new strains of this or other species, mainly in traditional fermented foods. Also, more fruits and vegetables fermented beverages should be developed using *Lactiplantibacillus plantarum* focusing on health promoting molecules.

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3. ***Enhancing the functional and nutritional aspects of a Jabuticaba (*****Plinia caulifora*****) juice by probiotic lactic acid fermentation***

3. FERMENTAÇÃO ÁCIDO LÁTICA PROBÓTICA DE UM SUCO DE JABUTICABA

O artigo "*Enhancing the functional and nutritional aspects of a Jabuticaba (*Plinia caulifora*) juice by probiotic lactic acid fermentation*." será publicado em uma revista de alto impacto da área de ciência de alimentos e nutrição.

*Enhancing the functional and nutritional aspects of a Jabuticaba (*Plinia caulifora*) juice by probiotic lactic acid fermentation*

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Abstract

The interest in probiotic fermented drinks have been increasing due to their benefits to human health, such as improvement in glycemic response and antioxidant status, and the modulation of the anti-inflammatory reactions and intestinal microbiota. However, considering the increasing public awareness of lactose intolerance and the tendency towards veganism, alternatives to the conventional dairy probiotic products have been claimed. Thus, fruit juices rich in phenolic compounds and fibers emerges as possible substrate of interest for fermentation by lactic acid bacteria. After this process, the product shows compounds with greater antioxidant, anti-inflammatory, anti-diabetic and immunomodulatory action. In this study, jabuticaba (*Plinia jaboticaba*) juice was anaerobically fermented by *Lactobacillus acidophilus* LA-5 during 72h at 37°C. Samples were taken every each 24h. Total phenolics, pH, sugars, organic acids, antioxidant activity, and viable cell count of microorganisms were analyzed before, during and after fermentation. The results indicated that the bacteria were able to significantly increase antioxidant activity through FRAP, ORAC, ABTS and TPC methods. Jabuticaba juice proved to be a good medium to *Lactobacillus acidophilus* growth since viable cells reached 4×10^7 CFU/mL at the end of fermentation, giving it probiotic characteristics. In conclusion, the lactic acid fermentation improved the functional features of jabuticaba juice.

Keywords

Probiotic, Antioxidant Activity, Fermented Food, *Lactobacillus*, Jabuticaba

1. Introduction

Fermentation is an ancient preservation technique in which desirable microorganisms (MOs) grow while convert food components, creating new flavors, textures, and improving nutritional and digestible aspects of food (Marco et al., 2021). This process can be done by probiotic MOs, which is defined as live microorganisms which when consumed in adequate amounts, confers health benefits to the host (Hill et al., 2014). Probiotic lactic acid bacteria (LAB) are largely used to ferment dairy beverages such as yogurt and kefir, being a good source of probiotics to the diet (Van Wyk, 2019). Nonetheless, the attention towards fermented non-dairy beverages has raised due to recent consumption tendencies (e.g., awareness regarding sustainability aspects of food systems, lactose intolerance and veganism) (Aschemann-Witzel et al., 2020; Ayed et al., 2020).

The consumption of fruits and vegetables have been linked with minor probability of developing non-communicable chronic diseases, such as diabetes type 2, cancer, cardiovascular and neurodegenerative diseases, and improve of cognitive function (Chikara et al., 2018; Medina-Remón et al., 2018; Miller et al., 2017). These benefits can be attributed to the antioxidant capacity of fruits and vegetables coming from their compounds such as phenolic compounds, carotenoids, and vitamins (Xu et al., 2017). The lactic acid fermentation of fruit juices can improve the antioxidant activity (AA) due to biotransformation and releasing of bound phenolic compounds by the enzymatic machinery of LAB (Filannino et al., 2018) . This mechanism could indicate that this type of product can be even better for the reduction of the risk for develop non-communicable diseases. Furthermore, lactic acid fermentation of a fruit juice can produce short chain fatty acids (SCFA), B- group vitamins, gamma-aminobutyric acid (GABA), exopolysaccharides (EPS) and be a good carrier for probiotic bacteria, all characteristics related to mental and body health (Szutowska, 2020). Moreover, fruit and vegetables juices fermented by a known LAB probiotic strain can be considered a synergistic synbiotic product (Swanson et al., 2020) and a plant polyphenol-based second-generation symbiotic according to Sharma & Padwad (2020).

Jabuticaba (*Plinia jaboticaba*) is a native berry from Brazilian Atlantic Forest. Similar to other dark-colored fruits such as blueberries and grapes, jabuticaba is characterized by high content of polyphenols including flavonoids, ellagitannins and anthocyanins, specially cyanidin-3-glucoside, which gives purple color to the fruit peel (S. Wu et al., 2012). Also, jabuticaba is rich in vitamin C, dietary fiber, minerals, and simple sugars (Geraldi et al., 2020). Its polyphenols are recognized as to have strong antioxidant potential in blood plasma, improve the metabolism of glucose and lipids and exert anti-mutagenic properties. These characteristics are related to an increase in the prevention of non-communicable diseases (NCD) such as cancer, hypertension, and diabetes (Lenquiste et al., 2019). Jabuticaba is a high perishable food, so its processing, like lactic acid fermentation, it is desirable to increase the consumption of the fruit and reduce food loss and waste (Plaza et al., 2016a; Ruiz Rodríguez et al., 2021).

Considering these aspects, this study aimed to develop a probiotic vegan product by using a strain of *Lactobacillus acidophilus* (specie that has resistance against low pH) for fermenting jabuticaba juice. The parameters monitored during fermentation were probiotic growth, organic acid production, AA, pH and sugar content.

2. Material and Methods

2.1 Microbial strain and activation

Lactobacillus acidophilus strain LA5 was purchased from Christian Hansen Ind. e Com. Ltda (Valinhos, SP, Brasil) and stored at -80°C in Man Rogosa Sharp (MRS) broth. 1g of bacteria (10^6 CFU/g) were activated in 900mL MRS broth added with 1.5% (w/v) of bile salts (HIMEDIA LABORATORIES PVT. LTA., India). After 48h under anerobic conditions at 37°C, each 100mL of the resulting suspension was added to another 900mL of MRS broth added with 1.5% of bile salts and incubated at 37°C for 48h. Then, the biomass was collected by centrifugation (4000 rpm, 10 min, 20°C) and washed with sterilized water. The process was repeated until the final inoculum concentration reached 10^8 CFU/ mL.

2.2 Preparation of Jabuticaba Juice

The jabuticaba fruits (*Plinia jaboticaba*) were immediately frozen at -18°C after harvest and were unfreeze at room temperature before processing. The juice preparation followed the process described in Geraldi et al. (2020). Briefly, the jabuticaba fruits were selected by their state of matureness and appearance, washed in water, and sanitized with 150 mg/L chlorine solution for 10 min and rinsed again with water. The whole fruits were mixed with potable water (1:1 w/w) in an industrial blender (Skymsen, LS-02MB-N, Brazil) for 3 minutes and then sieved (1 mm). 150 mL of jabuticaba juice were added to two 250ml Schott flasks each and closed to guarantee to avoid has exchange. The juices were pasteurized using a water-bath at 80°C for 5 minutes and then cooled to room temperature.

2.3 Fermentation conditions of jabuticaba juice

Three milliliters (2%, v/v) of the suspension (10^8 CFU/ mL) of *Lactobacillus acidophilus* LA-5 was inoculated in the 150mL of pasteurized jabuticaba. The juice was incubated at 37°C and samples were aseptically taken in different times of fermentation (0h, 24h, 48h, and 72h). Cell count was done immediately after sample recovery and the remaining sample was stocked at – 18°C until use for organic acids, sugars, and AA analyses. The samples were taken in duplicate for each Schott flask.

2.4 Determination of probiotic count

Enumeration of *Lb. acidophilus* LA-5 was performed on MRS agar supplemented with 0.15% (v/v) bile salts at 37°C for 72 h under anaerobic conditions. Basically, 0.1 mL of samples were serially diluted at a ratio of 0.1 (v/v) with peptone water (10 g/L of peptone, 5 g/L NaCl, 3.5 g/L Na_2HPO_4, 1.5g/L KH_2PO_4) in tube tests. Then, aliquots of dilution 10^{-6}, 10^{-7}, and 10^{-8} were dispensed in Petri dish containing MRS agar. The plates were incubated at 37°C during.72h. After the incubation, the number of colonies forming unit was determined by counting plates with 30-300 colonies (CFU/ml).

2.5 Sugar Analysis

Sugars (glucose, fructose and sucrose) were monitored during different times of fermentation according to the method described by Pereira et al. (2018). Briefly, samples were mixed with ultrapure water (1:15, w/v) and then vortexed for 20s. Subsequently, samples were filtered through 0.22 μm PVDF filter membranes units (MILLEX® GV, Carrigtwohill, Ireland). A High-Performance Anion Exchange Chromatography (HPLC) coupled to Pulsed Amperometric Detection (HPAEC-PAD) system model DIONEX ICS-5000 (Thermo Fisher Scientific, Waltham, USA) was used to identify and quantify sugars. The compounds were separated on a CarboPac PA1 column (250 × 4 mm, 10 μm particle size) using an isocratic mobile phase (0.12 mol/L NaOH) with 1.0 mL/min of flow rate, 30°C of temperature and 25 µL of volume injection. Sugars were identified in the samples by comparing the retention times of the standards and the samples. Calibration curves were constructed with commercial standards (0.25–12.50 μg/mL) to quantify the sugars in the juice. The content of individual compounds was expressed as g/L.

2.6 Physicochemical parameters

The pH value was determined utilizing a digital pHmeter.

2.7 Organic acids analysis

Lactic, succinic, tartaric, malic, citric, and acetic acids were monitored during fermentation by LC-10ADVP (Shimadzu Company, Japan). The mobile phase was KH_2PO_4 (0.1 mol/L, pH 2.0). An Agilent TC-C18 chromatographic column (250 x 4.6 mm i.d., 5 mm) kept at 22°C was used for the separation of the organic acids. The injection volumes were 20µL and the flow rate was 1 mL/min. The content of each organic acid was expressed as g/L. Each organic acid in the juice was quantified by comparing its peak area against the standard curve obtained with seven points of different concentrations of each standard compound.

2.8 Antioxidant activity and Phenolic Content

2.8.1 FRAP assay

The FRAP assay measures the antioxidant potential in samples through the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants present in the samples (Benzie & Strain, 1998). The FRAP reagent was prepared using 0.3 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ in 40 mmol/L HCl solution and 20 mmol/L FeCl3 (at proportions of 10:1:1).

The sample or standard solutions (7.5 μL), water (22.5 μL), and FRAP reagent (225 μL) were mixed and incubated at 37 ◦C for 30 min. Using a blank containing FRAP reagent as a reference, absorbance at 595 nm was determined at 30 min. The samples and Trolox standards were read at 595 nm, and the results were expressed as mg TE/L.

2.8.2 ORAC assay

The ORAC method (Ou et al., 2013) measures the decay of the fluorescence because of loss of conformity when undergoing oxidative damage. ORAC assay was carried out by adding phosphate buffer (PB pH 7.4)-diluted juice or standard solutions (25 μL), PB-diluted fluorescein (150 μL), and AAPH [2,2′-Azobis (2-amidino- propane) dihydrochloride] (25 μL) to black microplates. The microplate reader with fluorescent filters was defined as follows: excitation wave- length, 485 nm; emission wavelength, 520 nm. Trolox was used as standard, and ORAC values were expressed as mg Trolox equivalent per L (mg TE/L) by using the standard curves. The net area's linearity under the curve was checked for the samples, and the fluorescence readings were used to make the appropriate calculations.

2.8.3 ABTS assay

The ABTS radical scavenging capacity was determined according to Wootton-Beard et al., (2011) with slightly modifications. Briefly, 1mL of a 7mM of ABTS solution was mixed with 17.6 μL of a 140mM solution of potassium persulphate and kept in the dark for 16 hours. After that, the solution was diluted to an absorbance of 0.9-1 at 734nm. Then, 3 mL of the solution was mixed with 20 μL of standard (Trolox), ethanol or sample and incubated for 20 min in the dark at room temperature. Results were expressed as mg Trolox Equivalent/L of juice.

2.8.4 Total Phenolic Content

TPC was determined by Folin-Ciocalteu method as described by Swain & Hillis (1959) with modifications. Basically, 0.2 mL of the sample were mixed with 1.5 mL of Folin-Ciocateau reagent diluted in water (1:10 v/v). After 2h hours in the absence of light and room temperature, the absorbances of standard curve and samples were read at 725 nm. The results were expressed as milligrams of gallic acid equivalents (mg GAE) per L of fermented juice.

2.9 Statistical Analysis

The experiments were conducted in duplicate. For each Schott flask, two samples were taken. Each sample was analyzed in triplicate. The data were analyzed using Excel 2016

(Microsoft, USA). One-way analysis of variance (ANOVA) followed by T test were used to determine significant differences ($p<0.05$) between different times of fermentation. The results were expressed by the mean ± standard deviation.

3. Results and Discussion

3.1 Probiotic Growth and pH

The kinetics of the probiotic bacterium growth and pH value were measured during 72h of fermentation and the results are displayed in **Figure 1**. The initial bacterial cell count was $1.8 \pm 0.1 \times 10^5$ CFU/mL, remained roughly the same in the first 24h (lag phase) and then raised to 1.0×10^7 UFC/mL and 4.0×10^7 CFU/mL after 48 and 72h of fermentation, respectively. This result is in agreement with Mousavi et al., (2013) that also find no significant difference in the concentration of *Lactobacillus acidophilus* after 24h of fermentation of a pomegranate juice with pH 3 but noticed a transition to the log phase during 24h and 48h of fermentation, reaching 3.9×10^8 CFU/mL after 48h. On the other hand, Espirito-Santo et al., (2015) reported significantly increase in probiotic cell count during the first 8 hours fermentation of apple, grape and orange juices (pH 3.7, 3.4, 3.6 respectively) by different LAB. At the end of fermentation, the juice achieved a cell density of 4×10^7 CFU/ml. As the serving size of the juice is 200ml, the juice reached 8×10^9 CFU per serving, what can be considered a probiotic drink according to some regulatory organizations like the Italian Ministry of Health and Health Canada (Hill et al., 2014) and a probiotic fermented food according to Marco et al., (2021). Also, this result suggests that jabuticaba juice is a good medium for probiotic growing without any needs of supplementation.

In parallel, pH constantly dropped during fermentation, from an initial pH of 3.56 ± 0.03 to 3.198 ± 0.08 after 72h of fermentation. This result agrees with the findings of Sheng et al., (2022) that also noticed slight changes in pH during 48h fermentation of red grape juice (initial pH = 4) with *Lactobacillus acidophilus*. A low initial pH of the juices was reported to extend the lag phase (Mousavi et al., 2013).

Although the probiotic characteristics are defined to strain level, there are some health benefits and technological aspects that all bacteria from the specie *Lactobacillus acidophilus* have in common. This specie presents high tolerance against low pH and bile, adhesion to the human enterocytes, improvement in host lactose metabolism and immune system, and production of antimicrobial compounds (Bull et al., 2013). Furthermore, regulation of intestinal transit, normalization of perturbed microbiota, increase in the turnover of enterocytes, enhancement of mucosal barrier function, modulation of immune system, and competitive exclusion of pathogens are health benefits shared among all probiotics (Hill et al., 2014). In addition, probiotics were extensively investigated in past years in the modulation of sugar and cholesterol metabolism (Fu et al., 2020) and in the beneficial effects in mental health (e.g., decreasing depression and anxiety symptoms) (Ansari et al., 2020; de Araújo & Farias, 2020).

Lactobacillus acidophilus LA-5 was able to produce, bio transform and/or release bioactive molecules during the fermentation, such as acetic and lactic acids, increased the total phenolic compound content, and antioxidant activity as will be commented next.

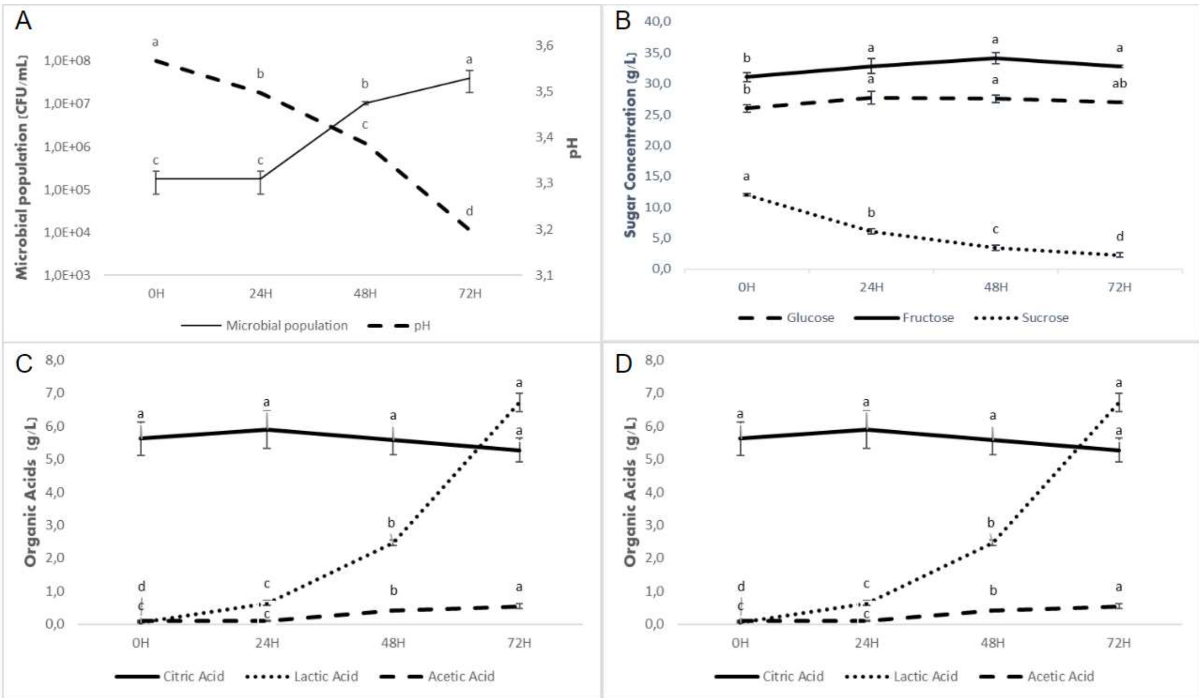

Figure 1. A) *Lactobacillus* growth and pH variation during fermentation of jabuticaba juice**.** **B)** Kinetics of sugar consumption during the fermentation **C)** Kinetics of citric, lactic, and acetic acid content during the fermentation **D)** Kinetics of succinic, malic, and tartaric acid content during fermentation. Different letters significantly refer difference ($p<0.05$). Error bars indicated the standard deviation of each sample.

3.2 Sugars and Organic acids analysis

The kinetics of glucose, fructose and sucrose consumption is displayed in **Figure 1B)**. *Lactobacillus acidophilus* decreased the concentration of sucrose, while the fructose and glucose contents presented a little variation during fermentation. The bacterium was able to reduce the amount of sucrose from 12,03 ± 0,19 g/L to 2,26 ± 0,37 g/L in the end of 72h of fermentation. Glucose and fructose significantly ($p < 0.05$) increased during the first 24h of fermentation, due to the hydrolysis of sucrose, and did not reduce significantly until the end of the 72h fermentation. In the last 24h of fermentation, with sucrose levels starting to extinguish, the reduction of sucrose content was lower (3.46 ± 0,44 g/L to 2.26 ± 0,37) with no significant difference in glucose or fructose contents, but with four times increase in cell count (Figure 1A).

LAB are capable to produce or to use organic acids as carbon source in hostile environment as low pH's values (Garcia et al., 2020). Therefore, lactic, citric, malic, succinic, tartaric, and acetic acids contents were monitored during fermentation and are displayed in Figure 1C) and 1D). As expected, lactic acid was not detected in the beginning of the process and reached 0.633 ± 0.075 g/L, 2.459 ± 0.08, and 6.71 ± 0.28 g/L after 24h, 48h, and 72h of fermentation, respectively. Lactic acid is the main organic acid produced by LAB during fermentation and responsible for the pH drop in the process. Furthermore, lactic acid could be metabolized to butyric acid by the gut microbiota (Bourriaud et al., 2005), a bioactive organic acid.

Before fermentation (0h), jabuticaba juice presented as major organic acids citric, succinic, and malic acids. These results are in agreement with the findings of Neves et al. (2021) and Lima et al. (2011) that also reported these organic acids as the most abundant in jabuticaba. In another study, researchers found a relation between citric and malic acid of 4.45 (de Andrade Neves et al., 2022). This result is very similar with this present study that shows a 5,91 ratio between citric and malic acid. On the other hand, a little amount of succinic acid was found in that study (0.08 ± 0.02 g/L) compared to the results present in this study (1.05 ± 0.197 g/L).

Some LAB, such as *Lactobacillus acidophilus*, are capable to use citric acid as substrate to growth in acidify medium, as demonstrated by Mousavi et al. (2013) during the fermentation of pomegranate juice with *Lactobacillus acidophilus* LA-5 in which citric acid content dropped from 60g/L to 15.5 g/L after 72h fermentation. Therefore, it was not observed in this study since the citric acid content did not change significantly during fermentation. Another study showed that *Lactobacillus acidophilus* did not metabolize citric acid during the fermentation of apricot juice during 24h (Bujna et al., 2018). However, succinic acid and malic acid suffered significantly changes ($p<0.05$) in their concentration during the fermentation process, result that agrees with the findings of this present study.

In the unfermented juice (0h), succinic acid concentration was 1.05 ± 0.19 g/L. This value did not significantly change ($p<0.05$) during the first 48h, but significantly dropped ($p<0.01$) during the last 72h of fermentation. This result suggests that *Lactobacillus acidophilus* used succinic acid as carbon source in this period. This finding is in agreement with Yang et al. (2022), that also reported a reduction in succinic acid content during the fermentation of Fuji apple juice by *Lactobacillus acidophilus* LA-5 from 160.7 ± 8.3 mg/L (0h) to 136.0 ± 1.1 mg/L (72). Curiously, after 12h of fermentation succinic acid decreased to 113.6 ± 14.9 mg/L, value significantly ($p<0.05$) lower than the concentration after 72h. In the same line, Fonseca et al. (2021) demonstrate the ability of two LAB strains (*Lactiplantibacillus plantarum* CCMA 0734 and *Lacticaseibacillus paracasei subsp. paracasei* LBC-81*)* to degrade succinic and malic acid during the fermentation of acerola, passion fruit and jelly palm juices.

Malic acid content also changed during the fermentation process. In the first 24h, the level of malic acid increased significantly ($p<0.01$) from 0.95 ± 0.03 g/L to 1.40 ± 0.20 g/L. Between 24 and 72h the level of malic acid dropped significantly ($p<0.01$) to 0.39 ± 0.02 g/L and did not change significantly ($p<00.5$) until the end of the process. The production and degradation of malic acid during different times of lactic acid fermentation was reported by Yang et al. (2022). *Lactobacillus acidophilus* was capable to significantly decrease ($p<0.05$) the content of malic acid during the first 48h of fermentation (1166.5±14.7 mg/L to 28.9±2.9 mg/L), but also significantly increase the amount of malic acid from 48h to 72h in 59%. In another study, researchers reported a significantly ($p<00.5$) increase in the amount of malic acid during 48h of jujube juice fermentation by *Lactobacillus acidophilus* (from 1282.1 ± 1.9 mg/L to 1896.4 ± 2.4 mg/L) (Zhang et al., 2022). In the same study, a strain of *Lactobacillus plantarum* reduced the initial amount of malic acid to 658.3 ± 4.4 mg/L in the same juice and same fermentation parameters, which shows the difference in organic acid metabolism by different LAB. On the other hand, Chen et al. (2018) reported a significantly increase ($p<0.05$) from 53.2 ± 2.8 mg / 100mL to 77.6±3.5 mg/100mL of malic acid during the fermentation of papaya juice by *Lactobacillus acidophilus*.

Acetic acid is the main shorty chain fatty acid (SCFA) found in the human microbiome and human feces, and the concentration of circulating acetic acid is negatively associated with fasting free fatty acids, triacylglycerol and appetite hormone called GLP-1 (Müller et al., 2019). Also, acetic acid is the main organic acid found in vinegar and its health properties could be due to this acid. It is known that the consumption of vinegar with a high-carb meal can reduce the overall glycemic index of the meal (Frias et al., 2016). Acetic acid is also considered a powerful anti-bacterial against the most common pathogenic bacteria such as *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* (Ryssel et al., 2009), which is important to the safety of the juice. On the other hand, a high increase in the acetic acid concentration during the juice fermentation can cause an off flavor in the product, increasing its rejection by the population (Szutowska, 2020).

The fermentation of jabuticaba juice improved the content of acetic acid from 0.103 ± 0.009 g/L (0h) to 0.544 ± 0.065 g/L (72h). This result is also higher than the one published by Mousavi et al. (2013) that found an increase in the acetic acid content during the fermentation of pomegranate juice by *Lactobacillus acidophilus* LA-5. After 72h, researchers reported an increase of 0.35 g/L in acetate concentration, but with high propionate production. *Lactobacillus acidophilus* also was capable to produce. In another study, researchers reported a production of 1.9 g/L of acetic acid after 24h fermentation of a pineapple juice by *Lactobacillus acidophilus* LA-5 (Nguyen et al., 2019). In the same study and parameters, *Bifidobacterium lactis* Bb12 and *Lactiplantibacillus plantarum* 299V were able to produce 1.3 g/L and 1.0 g/L of acetic acid after 24h of fermentation. In this line, Bujna et al. (2018) investigated the effect of four different strains in the lactic acid fermentation of an apricot juice. *Lactobacillus acidophilus* was the greatest productor of acetic acid, reaching 1.8 g/L after 24h of fermentation at 37°C. The pH of the initial juice made by (Nguyen et al., 2019) and (Bujna et al., 2018) were pH 6.7 and pH 6.1, respectively. Meanwhile, Mousavi et al., (2013) and this present study reported pH 3 and pH 3.56, respectively. The pH looks to play a key role in the metabolism of these organic acids during lactic acid fermentation. Also, the LAB metabolism of organic acids is intrinsically dependent of the genus, specie and strain of LAB and also, as described by Zalán et al. (2010), the medium. The researchers emphasize that some LAB strains could change their fermentation profile depending on the type of medium. More studies are needed to develop distinct kinds of fermented juices with production of specific organic acids, mainly with bioactivity, as the SCFA.

3.3 Antioxidant Activity and Total Phenolic Content

The consumption of foods rich in phenolic compounds (e.g., fruits, vegetables and whole grains) is related to an increase of the prevention on the development of non-communicable chronic diseases such as diabetes, hypertension, and some types of cancer (Miller et al., 2017). The protecting effect of these compounds are related to their great antioxidant capacity mainly attributed to their ability to scavenge or inhibit nitrogen and oxygen species, stabilize free radicals by transferring electrons and activating antioxidant enzymes (de Araújo et al., 2021). The chronic ingestion of these compounds reduces free radicals on blood, reducing the production of inflammatory cytokines and creating a less favorable environment to the development of these diseases. However, the microbial conversion of phenolics is directly linked with higher absorption of these compounds (Di Lorenzo et al., 2021). This process can be done by the gut microbiota during digestion or by some food fermentation process like LAB fermentation, because these bacteria are capable to produce enzymes that modify the phenolic compounds profile of the plants such as tannin acyl hydrolase, glycosyl hydrolase, and phenolic acid reductase (Filannino et al., 2018). Some of these modifications in the phenolic profile are related with an increased overall AA of the food (Szutowska, 2020). The main enzymatic reactions that contribute to an increase in AA are the deglycosylation of flavonoid glycosides (e.g., biotransformation of anthocyanins into anthocyanidins), hydrolysis of condensed tannins into smaller phenolic acids (e.g., gallic acid, and pyrogallol), and releasing of bound phenolics of plant wall cells (Septembre-Malaterre et al., 2018).

Although jabuticaba is already a powerful antioxidant food with anti-mutagenic and glucose homeostasis regulation properties (Leite-Legatti et al., 2012; Plaza et al., 2016b), the lactic acid fermentation may improve these parameters. The ability of *Lactobacillus acidophilus* LA5 to increase the AA of the jabuticaba juice was investigated by ORAC, FRAP, ABTS and TPC methods and the results are displayed in **Figure 6**. These methods were chosen to consider different mechanisms of antioxidant compounds present in fresh or fermented juices.

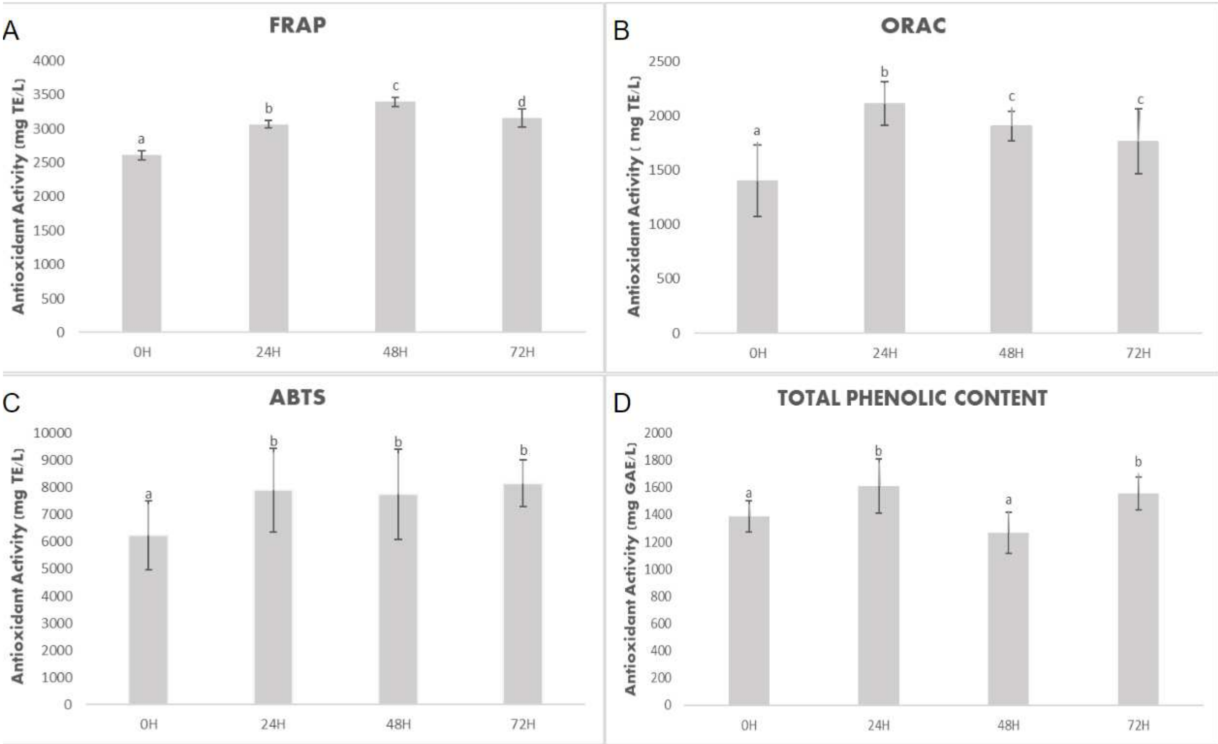

Figure 6. FRAP (A), ORAC (B), ABTS (C), and TPC (D) methods for antioxidant activity during different times of fermentation of jabuticaba juice by *Lactobacillus acidophilus* LA5. Error bars indicated the standard deviation from two independent samples. Columns with different letters represent significant difference ($p<0.05$).

3.3.1 ORAC

The ORAC method calculates the antioxidants' ability to interrupt the radical chain reaction through monitoring the inhibition of the oxidation of the peroxyl radical. ORAC method is one of the preferrable antioxidant methods because peroxyl radicals predominate in biological systems (e.g., lipid oxidation in humans, oxidation of food and beverages). For this reason, ORAC method is considered a reference method for AA assay (Munteanu & Apetrei, 2021).

The initial AA of the juice by ORAC method was 1,401 ± 327 mg Trolox Equivalent (TE)/L. This value is slightly higher than unpasteurized jabuticaba juice and slightly lower than heat treated jabuticaba juice made by the same method by Geraldi et al. (2020) (1,022 mg TE/L and 1,860 mg TE/L, respectively). All the fermentation times increased significantly ($p<0.05$) the AA in the juice. The highest AA was after 24h of fermentation increased 50%

the AA (2,110 ± 199 mg TE/L). This result is in accordance with Wen et al. (2020) that also found a significantly increase ($p<0.01$) in the AA by ORAC method during 24h fermentation of a litchi juice by a *Lacticaseibacillus casei* strain with initial bacteria concentration of 10^6 CFU/mL. After 48h of fermentation, jabuticaba juice improved 35% of its AA. It is also reported in literature an increase in AA by ORAC method during LAB fermentation of a mixed berry juice (acai, aronia and, cranberry) (Park et al., 2017). In this experiment, *Lactiplantibacillus plantarum* strain was capable to rise the AA by 28%. This result contrasts with the findings of Isas et al. (2020) that showed no significant difference ($p<0.05$) during 48h of cherimoya juice lactic acid fermentation by five different LAB strains. On the other hand, researchers reported 40% of decreasing in AA by ORAC during 12h fermentation of citrus (*Citrus reticulata* cv. Chachiensis) juice by *Lactobacillus fermentium* (Yu et al., 2015).

3.3.2 FRAP

FRAP method was done to measure the AA during different times of jabuticaba juice fermentation. In the control juice, the AA was 2,610 ± 68 mg TE/L or 1,043 ± 27 µmol TE / 100 mL. This finding is slightly lower than the result presented on other study conducted by Geraldi et al. (2020) that find 1392 µmol TE / 100 mL with the same preparation method of the jabuticaba juice. This is probably due to the difference of time between the production of these two juices. All fermentation times showed a significantly increase ($p<0.01$) in antioxidant activity compared to the control juice. After 48h, AA by FRAP method achieved its highest value (3397 ± 66 mg TE/L) and was significant different ($p<0.05$) among all other samples. This result is in contrast with the findings of Y. Wu et al. (2021) that did not find significant difference ($p<0.05$) after 24h of fermentation of blackberry and blueberry juices by two LAB and one *Bifidobacterium* (*Lactiplantibacillus plantarum* BNCC 337796, and *Streptococcus thermophilus* CGMCC 1.8748, *Bifidobacterium bifidum* CGMCC 15090) independently. On the other hand, researchers also found that *Lactobacillus acidophilus* significantly reduce the antioxidant activity of papaya juice by FRAP method after 18h of fermentation. In that study, Chen et al. (2018) showed a decrease of 20% after 48h of fermentation (5.68 ± 0.08 to 4.51 ± 0.16 mM $FeSO_4$).

Despite the results showed above, the increase in the AA by lactic acid fermentation of fruit and vegetables juices are frequent. Li et al. (2021) reported a significantly increase ($p<0.05$) in AA by FRAP method during the fermentation of two species of jujube fruit juice by *Lactobacillus acidophilus*. The highest values were observed after 12h and 24h of fermentation, and *Lactobacillus acidophilus* were able to increase 8.5% and 18% in AA of the two jujube juices varieties after 48h of fermentation. In agreement of this finding, other researchers also reported increasing in the AA activity by FRAP method by *Lactobacillus acidophilus* during the fermentation of Ginkgo biloba kernel juice, highlighting the superiority of this specie over a *Lacticaseibacillus casei* strain (Wang et al., 2019). In the same line, Nguyen et al. (2019) reported the ability of *Lactobacillus acidophilus* to increase the AA during the fermentation of a pineapple juice, recording a 12.7% increase after 24h of fermentation. Other researchers also reported increasing in AA by FRAP method during the fermentation of a fruit or vegetable by different LAB, such as sweet lemon juice by *Lactobacillus plantarum* (Hashemi et al., 2017), and tomato juice by the same LAB specie (Liu et al., 2018).

3.3.3 ABTS

The ABTS assay is widely spread method to determine the AA in lactic acid fruit juice fermentation. The result of AA by ABTS method showed a significantly increase during all times of fermentation. An increase of 26, 24 and 30% in AA was observed at 24, 48 and 72h respectively. However, there was no significant difference ($p<0.05$) between these three times. This result is in agreement with Yang et al., (2022) that reported an increase in AA by ABTS method during the fermentation of fuji apple juice by *L. acidophilus*, *L. casei* and *L. plantarum*. In another study, a group of scientists reported an increase in AA during the fermentation of a mulberry juice by three species of LAB independently, including *Lactobacillus acidophilus*. This one was responsible for an increase in the radical cation scavenging activity of ABTS ($ABTS^+$ -SA) from 70.42 ± 1.50 % in the control juice to 84.25 ± 1.04% after 36h of fermentation (Kwaw et al., 2018). In the same line, Qi et al. (2021) also portrayed the ability of a *Lactobacillus acidophilus* strain to significantly increase ($p<0.05$) the AA of a Chinese wolfberry juice during all fermentations time (12h, 24h, 36h, 48h). After 48h of fermentation, the bacterium was able to improve ABTS radical scavenging to nearly 50%, compared to 35% of the control group (0h). On the other hand, a research group also reported a significantly decrease in AA by ABTS method during the lactic fermentation of papaya juice by a strain of *Lactobacillus acidophilus* (Chen et al., 2018).

Despite *Lactobacillus acidophilus*, other studies demonstrated the efficacy of another LAB fermentation to improve AA by ABTS method. It can be highlighted the specie *Lactiplantibacillus plantarum* that improved the AA by this method during the fermentation of different fruit juices such as apple (Z. Li et al., 2019), blueberry (S. Li et al., 2021; Y. Wu et al., 2021), blackberry (Y. Wu et al., 2021), coconut (Kantachote et al., 2017), and pomegranate (Mantzourani et al., 2019; Pontonio et al., 2019; Valero-Cases et al., 2017). In general, lactic acid fermentation is a promising tool to enhance the AA by ABTS.

3.3.4 Total phenolic content

The Folin-Ciocalteu test is widely used to determine the total phenolic content (TPC) in clinical and nutritional studies, mainly in plant-derived foods and beverages (Munteanu & Apetrei, 2021). Thereafter, is a method with wide range use in the analysis to determine the AA of lactic acid fermented fruit juices. Like the other AA methods, variations in TPC depend on LAB strain, time, temperature, and substrate (Szutowska, 2020).

TPC improved significantly ($p<0.05$) from 1,389 ± 115 mg GAE/L in the control juice to 1,612 ± 199 mg GAE/L after 24h of fermentation. The result of phenolic content in the control juice is in accordance with previous report about the content of phenolic compounds in a jabuticaba juice by this method of preparation, reporting by Geraldi et al. (2020). Between 24h and 48h, TPC decrease significantly to 1,266 ± 152 mg GAE/L. In the end of fermentation, TPC reached 1,557 ± 119 mg GAE/L, result significantly higher than control juice and not significantly different than the 24h fermented juice. Both positive and negative variations in TPC during lactic acid fermentation were widely reported in literature. For example, C. Wu et al. (2020) reported a huge decrease in TPC during lactic acid fermentation of an apple juice by six different strains of probiotic, even the AA improved by FRAP method. Other authors also reported decreases in TPC during the fermentation of cherimoya juice by three *lactobacilli* strains after 48h.

On the other hand, it was reported that the fermentation of a pineapple juice by *Lactobacillus acidophilus* LA5 during 24h slightly increase TPC from 0.4 mg GAE/ml to 0.45 mg GAE/ml (Nguyen et al., 2019). Increase in TPC was also reported by other authors during the fermentation of a black carrot juice conducted by a LAB from the specie *Pediococcus acidilactici.* After 10 days of fermentation, the TPC improved 45.70% compared to day 0 (Manzoor et al., 2021). In accordance with this, Liu et al. (2018) reported the ability of two LAB (*Lactiplantibacillus plantarum* and *Lacticaseibacillus casei*) to improve the TPC during 48h fermentation of a tomato juice. Increase in TPC were also reported during the fermentation of pomegranate by *Lactiplantibacillus plantarum* strain. Curiously, TPC content continued dropping during the storage of non-fermented control juice, as opposed to the fermented one that improved the TPC during 28 days of storage (Mantzourani et al., 2019). Also, variations in TPC content also depend on initial substrate. For example, increase or decrease in TPC during the lactic acid fermentation of a potato juice by *Lacticaseibacillus casei* were related to the variety of the potato (Kim et al., 2012).

4. Conclusion

Lactic acid fermentation is an important strategy to enhance the nutritional aspects of fruits juices. *Lactobacillus acidophilus* LA-5 was able to significantly improve the AA of the juice by ABTS, FRAP, ORAC and TPC methods. The growth of the bacteria resulted in a probiotic drink (4 × 10^9 CFU/ 200mL) at the end of fermentation. Furthermore, the production of acetic and lactic acid, raised the bioactive attributes of the juice. This type of product should be further investigated in the next years due to the many health-related molecules present in the juice, unfamiliarity of the general population with this type of product, and unexplored market. New LAB strains and fruit and vegetables juices must be tested to achieved specific nutritional appeals.

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4. DISCUSSÃO GERAL

A revisão bibliográfica e os resultados obtidos a partir da fermentação ácido lática do suco de jabuticaba pela bactéria *Lactobacillus acidophilus* LA-5 evidenciam que o processo de fermentação ácido lática de sucos de frutas e vegetais por probióticos apresenta um grande potencial de consumo devido suas características funcionais, nutricionais, possibilidade de consumo entre vegetarianos estritos e inúmeras possibilidades de produção combinando a biodiversidade brasileira e a infinidade de cepas de BAL probióticas que podem ser aplicadas no processo. As variações de parâmetros de processo como temperatura, pH e concentração inicial do inóculo elevam as possibilidades de produção visando características nutricionais distintas. Após a fermentação ácido lática por probióticos, destacam-se os possíveis atributos nutricionais e funcionais: aumento do teor de vitaminas (em especial do complexo B), incorporação de probióticos, aumento da atividade antioxidante, produção de GABA (relacionado à saúde mental), EPS (característica prebiótica e tecnológica do produto) e ácidos graxos de cadeia curta (SCFA). Ao incorporar probióticos a um suco de frutas rico em compostos fenólicos, este suco ganha características simbióticas, ou seja, oferece tanto probióticos, quanto prebióticos e, portanto, possui um grande potencial modulador da microbiota intestinal (Swanson et al., 2020), fator relacionado a prevenção de DCNT (Hills et al., 2019). Em relação às vitaminas, esta produção pode assegurar o consumo diário de certas vitaminas que não tem fácil disponibilidade em alimentos de algumas localidades ou em certos tipos de dieta (ex: vitamina B12 no vegetarianismo e vegetarianismo estrito). Além disso, outro fator de destaque do processo fermentativo é a pré-digestão do alimento. As BAL atuantes durante o processo têm a capacidade de quebrar estruturas como oligossacarídeos, glúten e lactose, além de outros macronutrientes (carboidratos complexos e proteínas vegetais) melhorando a digestão e absorção do alimento (Castellone et al., 2021). Vale destacar que para cada benefício nutricional e funcional acima, a confirmação destas propriedades deve ser feita de maneira experimental.

Portanto, não restam dúvidas do ponto de vista nutricional que o resultado da fermentação ácido lática de sucos de frutas e vegetais é um produto com grande potencial funcional e nutricional. Estes aspectos são os pontos de partida de todo conhecimento rigorosamente científico da questão alimentar, porém o problema da alimentação é vastíssimo. Envolve aspectos culinário, gastronômico, econômico, social, higiênico, médico e até moral (Castro, 1932). Com isso, é necessário discutir como um produto como este deve ser introduzido à população, visto que apenas os seus benefícios nutricionais, sem um contexto alimentar, não explicitam um benefício claro ao elemento humano (Castro, 1968). Afinal, para problemas reais da alimentação, a pesquisa científica deve ser pensada de maneira inter e transdisciplinar (Boon and Van Baalen, 2019).

Para entender como se deve proceder a introdução de um novo alimento, primeiro é preciso entender o conceito de sistema alimentar. O sistema alimentar pode ser decomposto em três grandes seguimentos: a cadeia de alimentos (*farm to fork*), o ambiente alimentar e o comportamento do consumidor, todos esses influenciados por condutores políticos, econômicos, demográficos, socioculturais, ambientais e científicos (HLPE, 2017). Nos últimos 40 anos, a globalização (Santos, 2003) incidiu diretamente tanto nos seguimentos, quanto nos próprios condutores do sistema alimentar, criando verticalidades que podem ser observadas nos grandes centros do capitalismo e também em sua periferia, como em países como o Brasil. Estas verticalidades incluem baixa biodiversidade ofertada, grandes áreas de monoculturas, uso de agrotóxicos, grandes distâncias percorridas pelos alimentos e concentração de capital na mão de poucas empresas, desde máquinas e suprimentos para a agricultura até empresas multinacionais de alimentos e redes de supermercados (Belik, 2020; Bortoletto and Campelo, 2022; Malak-Rawlikowska et al., 2019).

Este padrão criado pela globalização é visível em alguns dados do sistema alimentar hegemônico. Aproximadamente 90% da alimentação global advêm apenas de 15 culturas, sendo trigo, milho e soja 50% da oferta (Abramovay, 2022), mostrando a fragilidade do sistema em oferecer biodiversidade aos pratos da população. A maioria dos alimentos consumidos no Brasil advém do varejo em geral, em que os supermercados representam 92,9% do faturamento e apenas 3 redes de supermercados detém 41,7% do mercado no Brasil, o que enfatiza a concentração de capital na comercialização de alimentos no país (Belik, 2020). A emissão total da produção de alimentos global contabiliza 30% do total de emissões de gases de efeito estufa, 70% do uso de água e 40% da superfície global , (Willett et al., 2019). Nos EUA, um alimento percorre uma média de 2400 km antes de ser consumido (Pimentel et al., 2008). Percorrer grandes distâncias no transporte de alimentos tem um efeito direto na emissão de gases de efeito estufa, já que contabiliza 20% de toda a emissão na cadeia de alimentos (Li et al., 2022), contabilizando transporte, produção e uso de terra. Ademais dos grandiosos números do sistema, 1/3 de tudo o que é produzido é perdido ou desperdiçado, chegando a 50% em frutas e vegetais. De 702 a 828 milhões de pessoas estão em situação de fome e 3,1 bilhões de pessoas não tem como pagar por uma alimentação saudável (FAO et al., 2022).

No entanto, ainda existe a ideia de que este sistema alimentar hegemônico global é a única forma de alimentar a população mundial. As evidências científicas mostram o contrário. Um estudo publicado na The Lancet mostra que este sistema alimentar hegemônico, além de não cumprir o seu papel na resolução dos problemas alimentares mundiais, é um modelo que interconecta e reforça as crises climáticas, as doenças crônicas não transmissíveis e a fome, criando a denominada Sindemia Global (Swinburn et al., 2019). Ou seja, a introdução de um novo produto dentro deste mesmo sistema alimentar pode agravar os problemas já existentes. Não obstante, é necessário pensar o resultado da pesquisa científica em tecnologia e ciência de alimentos dentro de uma ideia radicalmente diferente da posta hoje em nossa sociedade, do alimento como mercadoria (Machado et al., 2016). Ao analisar como a academia trata a ideia de alimento, Vivero-Pol (2017) conclui que o “alimento como bem particular” ou “alimento como *commodity*” é uma ideia dominante nos meios acadêmicos em contraste com “alimento como bem público” ou “alimento como bem comum”. O autor sugere que é urgente que a academia pense o alimento para além da ideia dominante para a resolução dos problemas alimentares globais. E para sair desta ideologia (Engels and Marx, 2007), é necessário olhar para a alimentação, e em especial neste trabalho para a fermentação ácido lática de plantas no Sul Global, dentro do seu conceito histórico.

A fermentação ácido lática é uma tecnologia altamente empregada há milênios no Sul Global, em países da África (Anukam and Reid, 2009; Rashwan et al., 2021), do Leste Asiático (Swain et al., 2014) e pelos povos tradicionais da América Latina (Penna et al., 2017; Ramos and Schwan, 2017). Sua escala de produção, tipo de fermentação e forma de comércio nessas localidades são baseadas em meios não hegemônicos. A fermentação nestas localidades é majoritariamente realizada a nível doméstico e em pequena escala, os processos são conduzidos por fermentação espontânea ou *back-slopping* e o seu comércio baseado em circuitos curtos de consumo, vendido em comunidades locais, ou produzido para consumo próprio (Chelule et al., 2010; Odunfa, 1988; Ramos and Schwan, 2017). Este modo de produção dos povos tradicionais do Sul Global ainda é utilizado por muitos pequenos agricultores nestas regiões. Estes pequenos produtores utilizam um modelo de agricultura baseado em alimentos como bem-comum, métodos agroecológicos, posse de terra menos androcêntricas e simbiose na relação com outros elementos naturais não humanos (Figueroa-Helland et al., 2018). Estas características do processo nestas localidades são contrárias às verticalidades, ou seja, podem ser definidas então como horizontalidades do sistema alimentar, e a sua adoção em outros espaços geográficos gera diferentes resultados no campo da economia, cultura e sociedade (Santos, 2003). A fermentação ácido lática não é apenas uma ferramenta da segurança alimentar e nutricional, porém da soberania alimentar destes agricultores, pois não apenas os garantem o acesso a uma alimentação saudável, mas também reforçam sua cultura, territorialidade e dão a esses povos o controle dos meios de produção de seus alimentos (Santos and Ortega, 2019). E é dentro deste conceito que o texto irá apresentar os benefícios da fermentação ácido lática, principalmente nas horizontalidades criadas pelos povos latino-americanos e africanos, como possíveis ferramentas para solução dos problemas criados pelo sistema alimentar hegemônico. Afinal, a formação do povo brasileiro se fez pelo entrechoque desses povos (Ribeiro, 1995).

Povos tradicionais fermentam plantas antes da descoberta dos microrganismos e do uso do aço, o que evidencia que a instalação de um empreendimento de fermentação em pequena escala ou a nível doméstico não necessita de grandes investimentos e nem equipamentos caros. Estes ainda podem ser compartilhados pelos produtores dentro de uma comunidade, cooperativa ou agroindústria familiar (Gazolla and Pelegrini, 2008; Marshall and Mejia, 2011). A fermentação ácido lática é um processo que aumenta o valor agregado do produto. Portanto, este tipo de empreendimento pode ser um gerador de renda para comunidades, aumentando a circulação interna de capital e levando ao desenvolvimento territorial regional (Battcock and Azam-Ali, 1998). Esse é um ponto que vai contra a lógica concentradora de capital do sistema alimentar hegemônico. Além disso, o emprego de um empreendimento fermentador é inclusivo. Diferente da agricultura tradicional, a fermentação geralmente não demanda um trabalho físico rigoroso. Com isso, pessoas com alguma incapacidade física e idosos podem se beneficiar desenvolvendo novas habilidades, resultando na produção de alimentos com alto valor agregado que podem ajudar a aumentar a independência financeira e a autoestima (Marshall and Mejia, 2011). Outro ponto é que a implementação destes empreendimentos na zona rural pode ser um atrativo para jovens permaneceram no campo, evitando uma fuga para cidades, um dos grandes problemas agrários da atualidade (Maïga et al., 2020) Os sucos fermentados por bactérias ácido láticas probióticas podem ser um produto a ser processado nestes empreendimentos, pois possuem um alto valor agregado devido aos seus benefícios funcionais e nutricionais.

Em relação aos microrganismos, a partir da obtenção inicial de uma cepa de BAL probiótica, esta pode ser reproduzida posteriormente a partir da técnica de *back-slopping*, (técnica amplamente empregada nos processos fermentativos do Sul Global) levando em consideração questões básicas de boas práticas de fabricação e higiene (Holzapfel, 1997; Odunfa, 1988; Ramos and Schwan, 2017). Esta técnica consiste basicamente em utilizar uma pequena quantidade de uma produção bem-sucedida previamente como inóculo da próxima fermentação. Esta reprodução de microrganismos leva a uma grande economia ao produtor, pois não há necessidade da compra destes microrganismos, o que diminui os custos de produção de sucos fermentados e aumenta seu acesso ao longo da cadeia. Este método também possibilita uma fermentação mais rápida e segura em relação à fermentação espontânea, além de garantir maior replicabilidade da qualidade do produto final (Boethius, 2016; Marco et al., 2021) Além disso, com a introdução de uma simples estufa no empreendimento familiar, é possível aplicar um processo de secagem para estocar este inóculo em temperatura ambiente e local arejado, assim como praticado em algumas regiões da África por secagem solar (Holzapfel, 1997).

O modelo hegemônico de produção agrícola foca na produção de poucas plantas em grandes áreas e a biodiversidade que o sistema oferece é limitada e considerada um dos maiores desafios deste sistema (Abramovay, 2022). A produção da agricultura familiar, pequenos produtores e povos tradicionais é que busca a biodiversidade para fornecer alimentos à população (Figueroa-Helland et al., 2018). Os índios Tupinambá e Guarani, por exemplo, exploravam intensamente a biodiversidade brasileira na produção de alimentos fermentados devido ao seu grande conhecimento botânico. Milho, arroz, mandioca, batata doce, cará, taioba, abóbora, banana, jenipapo, abacaxi, amendoim e caju eram plantas comumente usadas na fabricação do Cauim, uma bebida fermentada majoritariamente por BAL (Almeida et al., 2007; Noelli and Brochado, 1998). Este é outro ponto em que a aplicação da fermentação ácido lática é mais adequada dentro das horizontalidades do que das verticalidades do sistema alimentar brasileiro, pois pode levar biodiversidade para a mesa da população. As possibilidades da produção de sucos à base de plantas fermentados por BAL no Brasil têm um grande campo de exploração devido à grande biodiversidade brasileira. Frutas nativas brasileiras (Carvalho and Conte-Junior, 2021; Clerici and Carvalho-Silva, 2011; Neri-Numa et al., 2018; Reis and Schmiele, 2019) e PANC (Leal et al., 2018) são duas classes de plantas que apresentam grande potencial para a produção de sucos fermentados por BAL, criando produtos com atributos nutricionais e sensórias únicos. Não obstante, a quantidade de BAL a serem empregadas nesse processo são incontáveis. Apenas o antigo gênero *Lactobacillus* (há pelo menos mais 12 gêneros de BAL que podem ser empregados nesse processo, além do gênero *Bifidobacterium*) possuía em 2010 168 espécies (Ibrahim, 2016). Para cada espécie, há uma quantidade de cepas diferentes e os resultados em relação as transformações bioquímicas durante o processo de fermentação são dependentes de qual cepa for escolhida (Garcia et al., 2020; Szutowska et al., 2021). Neste trabalho, foi escolhida uma fruta nativa brasileira já conhecidamente com bom poder modular da microbiota intestinal em ensaios in vivo, O trabalho conclui que a atividade antioxidante da jabuticaba, atributo relacionado com sua grande quantidade de compostos fenólicos, teve um aumento significativo ($p<0.05$) da e o processo após 72h incorporou probióticos ao suco a uma concentração de 4 x 10^7 UFC/mL. Em uma porção de 200 mL de suco, este fornece 8 x 10^9 UFC, quantidade suficiente para o alimento ser classificado com um alimento fermentado probiótico (Marco et al., 2021).

A combinação de cepas de BAL probióticas como *starter cultures* para produção de bebidas tradicionais fermentadas majoritariamente por BAL pode ser uma boa ferramenta para a segurança do produto. Por exemplo, Väkeväinen et al. (2020) utilizaram cepas de bactéria das espécies *Lactococcus lactis* e *Pediococcus pentosaceus* como *starter cultures* durante a fermentação de uma bebida tradicional mexicana a base de milho, o *Atole agrio*. Os resultados obtidos mostraram que em comparação com a fermentação espontânea, o uso destas bactérias acelerou a redução do pH e diminui a contagem de bactérias da família *Enterobacteriaceae* após 6 horas de fermentação, aumentando a segurança microbiológica do produto. Na mesma linha, pesquisadores nigerianos documentaram a utilização de uma cepa de *Leuconostoc mesenteroides* na fabricação de *Agadagidi*, uma bebida fermentada tradicional a base de bananas maduras. Em comparação com a fermentação espontânea, a inoculação da BAL foi capaz de reduzir o conteúdo de anti-nutrientes, como fitato e oxalato (Oriola and Boboye, 2018). A adição de cepas probióticas a fermentações espontâneas de bebidas tradicionais brasileiras como o cauim, calugi, aluá e tarubá pode aumentar a segurança do processo, criar um produto com características funcionais e nutricionais distintas e ainda promover a difusão cultural destas bebidas, ainda pouco conhecidas e pesquisadas no Brasil. Além disso, não faltam exemplos de alimentos fermentados tradicionais latino-americanos (Penna et al., 2017) e africanos (Franz et al., 2014) para criação de novos processos de fermentação ácido lática a partir de substratos não comumente fermentados em território brasileiro, ofertando estes alimentos de alto valor nutricional e funcional e contribuindo para a difusão cultural de nossos povos. Infelizmente, existem poucos estudos sobre as bebidas fermentadas (majoritariamente por BAL) tradicionais da América Latina na literatura.

O Brasil tem um programa de alimentação escolar que é referência no mundo, o Programa Nacional de Alimentação Escolar (PNAE), que atende 40 milhões de crianças em todo o país (Chaves et al., 2020). A lei nº 11.947, de 16 de junho de 2009 determina a compra de no mínimo 30% do valor repassado de gêneros alimentícios diretamente da agricultura familiar local, do empreendedor familiar rural local ou de suas organizações (Amorim et al., 2020). No entanto, nem todas as cidades conseguem atender essa quantidade mínima. Na região Nordeste, por exemplo, em média as compras são de 20% do total do valor repassado e a incapacidade de produção, distribuição e armazenamento do produtor rural são parte do problema (Chaves et al., 2020). A fermentação ácido lática pode ser uma ferramenta importante para a agricultura familiar para esta finalidade já que, mas não apenas, é um processo de preservação de alimentos. Portanto, a fermentação de frutas, verduras, legumes e outras plantas pode ajudar principalmente no armazenamento e durante o transporte desses alimentos pelo produtor, assegurando maior controle de seu estoque e alcance do seu produto na cadeia (Marshall and Mejia, 2011). A fermentação ácido lática é um dos métodos de biopreservação mais empregados no mundo. As BAL podem produzir várias moléculas com potencial anti-patogênico como: peróxido de hidrogênio, antifúngicos (ex: propionato, alguns ácidos graxos, fenil-lactato, hidroxifenil-lactato), ácido acético, ácido lático, peptídeos bioativos, substâncias antimicrobinas (ex: reuterina, reutereciclina e diacetil) e bacteriocinas. A produção destes compostos está relacionada com a proteção do alimento contra os patógenos mais relacionados com a contaminação de alimentos como *Bacillus cereus*, *Salmonella* ssp. e *Escherichia coli* (Baschali et al., 2017; Licandro et al., 2020; Paul Ross et al., 2002). Outros pontos relacionados a segurança dos alimentos fermentados por BAL são os seus papeis na inativação de fatores anti-nutricionais, diminuição da caráter alergénico de proteínas vegetais, diminuição do conteúdo de aminas biogênicas e de micotoxinas (Licandro et al., 2020). Este benefício em potencial não foi explorado pela fermentação do suco de jabuticaba presente neste trabalho, mas a confirmação por estudos experimentais nas condições específicas é de grande importância para a aplicação da tecnologia na prática.

O valor agregado ao produto do agricultor pela fermentação ácido lática gera uma maior renda a este produtor. Esta renda pode ser convertida em uma ampliação do empreendimento de fermentação gerando maior volume de produção (Marshall and Mejia, 2011) ou mesmo numa melhora do estado nutricional do produtor, visto que a insegurança alimentar afeta 3 a cada 5 pessoas no meio rural (Maluf et al., 2022). A produção de bebidas fermentados por BAL probióticas pela agricultura familiar pode incidir diretamente sobre a qualidade nutricional de crianças e adolescentes pela política do PNAE. A obesidade tem crescido de forma exponencial nesse público. Apenas de 2008 a 2021, a obesidade infantil cresceu mais de 70% no Brasil (Lichotti and Valente, 2022). Nos sucos fermentados por BAL, a atividade antioxidante, probióticos, SCFA e os polifenóis estão diretamente associados à prevenção da obesidade e DCNT (Gille et al., 2018). O trabalho com o suco de jabuticaba, por exemplo, mostrou o bom crescimento da bactéria probiótica juntamente com significativo ($p<0.05$) aumento da atividade antioxidante comparada ao suco in natura por quatro métodos diferentes. A revisão da literatura sintetizada no capítulo de livro também concluiu que este incremento no conteúdo de moléculas e propriedades dos sucos relacionados à prevenção de DCNT é recorrente Os benefícios nutricionais gerados produções destes fermentados, obviamente, também podem ser utilizados pelos agricultores familiares, prevenindo o desenvolvimento de DCNT, que também cresce entre essa população (Saúde, 2019). Portanto, o investimento estatal na difusão desta tecnologia para os agricultores familiares pode gerar benefícios múltiplos no enfrentamento aos maiores problemas da alimentação global: fome e DCNT. Além do PNAE, a fermentação ácido lática também pode ser aplicada para aumentar a oferta de frutas, legumes e verduras em outros espaços públicos como feiras de rua, restaurantes populares e restaurantes universitários.

Agricultores familiares apontam que o número de pessoas que procuram seus produtos pelas redes sociais e aplicativos de mensagens vem crescendo. Nestes canais de venda, o ganho monetário final do produtor é maior, já que não há um atravessador entre o campo e a população (Feiden et al., 2020). O fato de um suco, como o desenvolvido neste projeto, poder apresentar o termo probiótico também é um diferencial do produto, já que, assim como os alimentos fermentados em geral, são produtos percebidos pela população como alimentos funcionais e saudáveis (Annunziata and Vecchio, 2013; Rojas-Rivas and Cuffia, 2020). Isto pode ser um diferencial para alavancar as vendas dos agricultores. Para além do termo probióticos, nos últimos anos pesquisadores sugeriram uma dose diária de microrganismos vivos na alimentação humana (Marco et al., 2020). A introdução de alimentos fermentados, como o suco proposto neste trabalho, dentro da lógica dos circuitos curtos de consumo (Paciarotti and Torregiani, 2021) também pode ajudar a fornecer essa quantidade diária de microrganismos vivos (Rezac et al., 2018) sem um gasto energético e ambiental grande durante o transporte e também sem grandes modificações dos microrganismos após obter o produto final, visto que o tempo entre produção e consumo nestes sistemas são muito menores. Diminuir as distâncias entre produção e consumidor, também é importante para redução das perdas de alimentos, já que o transporte de alimentos é uma de suas principais causas (Lipińska et al., 2019). A fermentação ácido lática também pode ser uma ferramenta importante para frutas e verduras que não são aceitas pelo consumidor por não possuírem tamanho e aparência desejadas, sendo direcionados para o processo de fermentação, o que também reduz as perdas e desperdícios destes gêneros alimentícios (Marshall and Mejia, 2011).

Por fim, espero que esta discussão sobre a aplicação da fermentação ácido lática em frutas e verduras seja vista como uma formulação de proposta para o enfrentamento do sério problema que é nosso atual sistema alimentar. Afinal, este é o papel da universidade pública. Um palco para livres discussões sobre projetos, ideias e soluções factíveis e politicamente viáveis a serem implementadas pelo poder público, visando a construção de um projeto nacional de desenvolvimento de médio a longo prazo que encare problemas nacionais, como é o caso da alimentação (Galeazzi, 1996).

5.CONCLUSÃO

Os sucos de frutas e vegetais fermentados por BAL probióticas apresentam um potencial nutricional e funcional. Atividade antioxidante, produção de moléculas bioativas (ex: SCFA, EPS e GABA), nutrientes (ex: vitaminas), pré-digestão dos macronutrientes, biotransformação de fenólicos e incorporação de probióticos são alguns dos benefícios mais recorrentes neste produto listados na revisão do trabalho. Os resultados obtidos com a fermentação do suco de jabuticaba confirmam um aumento significativo da atividade antioxidante por quatro diferentes métodos e a possibilidade de incorporação de probióticos em sucos, mesmo com baixo pH inicial, evidenciando esse produto como uma opção de fonte de probióticos não láctea. A partir da aplicação da fermentação ácido lática em sucos *in natura* é possível criar um método eficiente de preservação de frutas e vegetais, gerando produtos funcionais e consequentemente aumentando o valor agregado da matéria-prima. Com os resultados apresentados, espera-se uma maior exploração do potencial de produção de bebidas probióticas a base de frutas e vegetais e a aplicação desta técnica como importante ferramenta para solução de alguns problemas do atual sistema alimentar.

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7.ANEXOS

Anexo I – Cadastro do Patrimônio Genético

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº AAF84CA

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**AAF84CA**
Usuário:	**Gustavo Henrique Torres de Almeida Camillo**
CPF/CNPJ:	**366.782.228-65**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Plinia jaboticaba

Título da Atividade:	**COMPOSTOS BIOATIVOS EM SUCO DE JABUTICABA FERMENTADO POR Lactobacillus acidophilus**

Equipe

Gustavo Henrique Torres de Almeida Camillo	**FEA-UNICAMP**
Mário Roberto Maróstica Junior	**FEA-UNICAMP**
Juliano Lemos Bicas	**FEA-UNICAMP**

Data do Cadastro:	**10/11/2020 15:33:54**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **15:38** de **10/11/2020**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO · SISGEN

Anexo II – Autorização Elsevir para inserção do capítulo de livro

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